Depression and Anxiety in The Pregnant Omani Population in Relation to Their Fatty Acid Intake and Levels

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September 2023

This thesis is submitted for the degree of Doctor of Philosophy at

Imperial College London

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Statement of Originality

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise by reference in the text, and that this work has not been submitted for any other degree or professional qualification.

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Abstract

Introduction: Maternal depression during and after pregnancy is a worldwide public concern. Low FAs levels and intake in women during pregnancy were associated with a high rate of maternal depression and poor pregnancy outcomes. The study examines the association between vitamin levels, FAs intake and levels and prenatal depressive and anxiety symptoms and pregnancy outcomes among pregnant Arabic-speaking women in Oman.

Methodology: In 300 pregnant Omani women, level of depression and anxiety is assessed at the 8-12 and 24-28 weeks of pregnancy using the Arabic version of (EPDS). Seafood and the omega-3 FAs intakes of pregnant women has been quantified by using a validated (FFQ). Maternal Vitamins levels were assessed, and FAs analysis of erythrocytes was carried out using the method of Folch et al.

Results: Maternal depression and anxiety symptoms (30.5% and 26.1%) were associated with low fish consumption and omega-3 FAs intake with depressive and anxiety symptoms ($p = 0.01$), Women with antenatal depression or anxiety symptoms had a lower erythrocyte concentration of arachidonic acid (20:4 n-6), ($p = 0.01$), total omega-6 FAs, ($p = 0.03$), docosahexaenoic acid (22:6 n-3) ($p = 0.03$), docosapentaenoic acid (22:5 n-3) ($p = 0.04$), eicosapentaenoic acid (20:5 n-3) ($p= 0.005$), total omega 3 FAs ($p = 0.005$), omega-3 index ($p = 0.01$) and (AA+DHA)/MUFAs ($p = 0.01$), but a higher omega-6/omega-3 ratio ($p = 0.04$), compared to healthy pregnant women. These findings did not change after adjusting for potential confounders. The study outcomes show a significant relationship linking fish and omega-3 FAs intake with GDM PE, and birthweight centiles ($p = 0.031$).

Conclusions: Maternal fatty acids exert a favourable effect on vital perinatal health outcomes. Fish and seafood intake or omega-3 FAs supplementation are highly recommended for women during pregnancy to ensure the well-being of both the mother and fetus.

Acknowledgement

I am profoundly grateful to Almighty Allah for bestowing upon me the wisdom, confidence, strength, and perseverance that enabled me to successfully complete my studies. Without His boundless mercy and grace, this accomplishment would not have been possible.

I wish to express my heartfelt appreciation to my esteemed supervisors, Professor Michael Crawford and Professor Mark Johnson, for placing their trust in me, offering invaluable guidance, and providing unwavering support throughout my academic journey. Your mentorship has been a true inspiration, and I aspire to follow in your route. Thank you sincerely.

My deepest gratitude goes to Dr. Yiqun Wang, whose mentorship as a laboratory scientist has been instrumental in shaping my intellectual and practical growth. Your expertise, patience, and unwavering motivation have turned this project into a reality. I am truly grateful for everything.

I extend my thanks to Dr. Natasha Singh and Professor Dimitris Grammatopoulos for their thoughtful reviews and beneficial guidance during the early and late stages of my project. Your insightful advice and constructive comments have been immensely valuable in setting up the clinical study.

I also want to express my gratitude to my fellow lab colleagues for their unity, exemplary behaviour, and amicable support, which created a collaborative and productive environment. Special appreciation goes to my wonderful friends and colleagues, Noushin Masoudi and Annie Belle Sassine, for their exceptional guidance and training in enhancing my laboratory skills for analysing fatty acids and conducting precise experiments. Your constant assistance in resolving my queries and overcoming obstacles has been invaluable. The memories of our time spent together in the lab, working on the lipid extraction process and waiting for the GC outcomes, will forever be cherished.

To my friend, Dr Mohammed AL Meqbaly, I extend my sincere thanks for your unwavering assistance and expertise in data analysis, which greatly facilitated the writing process. Thank you for being a true friend and supporting me throughout my PhD journey. Special

thanks also to Professor Samir AL Adwai from Sultan Qaboos University for believing in me and providing constructive advice and guidance for my project.

Above all, I want to express my deepest gratitude to my family for their unwavering support and constant encouragement over the years. Their sacrifices have enabled me to achieve my goals and obtain an exceptional education. I am immensely grateful for their unwavering belief in me and for standing by my side at every step of this journey. Your prayers, motivation, encouragement, and support have played a pivotal role in turning my dreams into reality. Thank you from the bottom of my heart.

I extend exceptional thanks to the staff at Al Buraimi Hospital in the Ministry of Health in Oman for their unwavering assistance during data and sample collection and for their valuable contributions during the setup and preparation phases of this research. I am also deeply grateful to the Ministry of Higher Education, Research, and Innovation in Oman for funding my research costs and making this project possible. Thank you for awarding me this scholarship, which has helped me achieve my life's objectives.

Lastly, I want to extend my heartfelt thanks to all the mothers who participated in this study and generously donated their blood for research purposes. Your contributions are highly valued and deeply appreciated.

To everyone mentioned above and all others who have supported me along this journey, I offer my heartfelt thanks. Your encouragement and assistance have been instrumental in my success.

Thank you all!

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Abbreviations

AA	Arachidonic acid
ADA	Adrenic acid
ADHD	Adult attention-deficit/hyperactivity disorder
AHA	American Heart Association
ALA	Alpha-Linolenic acid
ALSPAC	Avon Longitudinal Study of Parents and Children
C14:0	Myristic acid
C15:0	Pentadecanoic acid
C16:0	Palmitic acid
C17:0	Heptadecanoic acid
CVD	Cardiovascular disease
CE	Cholesterol esters
CS	Caesarean section
DGLA	Dihomo-γlinolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DSM III	Diagnostic and Statistical Manual of Mental Disorders III criteria for depression”
EFAs	Essential FAs
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EPDS	Edinburgh Postpartum Depression Scale
FAs	Fatty acids
FAME	FAs methyl ester
FDA	Food and Drug Administration
FFQ	Food-frequency questionnaire
FR	Food records
GDM	Gestational diabetes
HADS	Hospital Anxiety and Depression Scale
HDRS-SF	Hamilton Depression Rating Scale
HPA	hypothalamic-pituitary-adrenal

IQ	Intelligence quotient
ISSFAL	International Society for the Study of FAs and Lipids
LA	Linoleic acid
LBW	Low birth weight
MA	Mead acids
MBI-SS	Maslach Burnout Inventory (Student-Survey)
MDD	Major Depressive Disorder
MeHg	Methylmercury
MOH	The Ministry of Health in Oman
MUFAs	Monounsaturated FAs
NICE	National Institute for Health and Care Excellence
PE	Preeclampsia
PHCs	Primary healthcare clinics
PHQ-9	Patient Health Questionnaire
PL	Phospholipids
PTB	Preterm birth
PUFAs	Polyunsaturated FAs
RBC	Red blood cells
RCTs	Randomised controlled trial
SGA	Small for gestational age
T2D	Type two diabetes
TG	Triglycerides
UK	United Kingdom
USFAs	Unsaturated fatty acids
WHO	World Health Organization

Chapter 1: Introduction

1.1 Depression Illness

Emotional disorders such as depression have several psychological and physical effects that diminish an individual's functionality and reduce their quality of life (1). The affective symptoms of depression may include restlessness, impaired memory, helplessness, emptiness, pessimism, loss of interest in pleasurable activities, anxiety, worthlessness, persistent sadness, poor concentration, guilt, irritability, hopelessness, and diminished ability in decision-making. Such despondency can sometimes progress to suicidal ideation (2).

Depression symptoms can appear in conjunction with several physical sicknesses, including fatigue, headaches, pains, decreased energy, non-specific aches, cramps, and irritable stomach (3). Excessive sleeping or insomnia, or eating disorders such as anorexia, are also common in depressed patients (1). Research shows that such functional impairments can lead to dependency and disability (4).

World Health Organization (WHO) studies estimate that in 2000, around 340 million people suffered from depression globally (5). They also predicted that depression would become more prevalent than other debilitating disorders, such as ischemic heart disease, which is expected to affect the quality of life significantly by 2020 (6). Furthermore, a study by the WHO, including more than 14 countries worldwide, indicated that depressive illness is the most commonly-detected mental health disorder in primary healthcare clinics (PHCs) (7).

Limited research shows the incidence of depression and anxiety symptoms among people in Oman. In a nationwide student population, Jaju et al. and Afifi et al. (8,9) indicated that 16% to 17% of the study sample were depressed, as measured by the World Health Organization Composite International Diagnostic Interview. Al-Salmani et al. (10) observed depression at a rate of 8% among clients seeking consultations at PHCs using A Patient Health Questionnaire (PHQ-9).

Moreover, Al-Busaidi et al, (11) reported that approximately 28% of medical trainees in Oman suffer from depression using A Patient Health Questionnaire (PHQ-9). In addition, a recent study indicates that around 25% of medical students in Oman display depressive symptoms using The Maslach Burnout Inventory (Student-Survey) (MBI-SS) (12). Among the geriatric population attending PHCs, (13) reported that approximately 17% of their cohorts were depressed using The Arabic version of the Geriatric Depression Scale (15 questions). Moreover, in another study of patients attending a dermatology clinic in Muscat, 24% of the patient sample had depressive symptoms using A Patient Health Questionnaire (PHQ-9) (14).

In Oman, a study revealed that about 28% of individuals with cancer had depression using the Hospital Anxiety and Depression Scale (HADS). (15), while a recent study conducted on adults with Type two diabetes (T2D) in Oman revealed that about 26% experienced clinical depression using A Patient Health Questionnaire (PHQ-9) (16). Yet, there are very little data about the treatment outcomes of people with depression.

Table 1-1 Mental Health scale uses:

Mental Health scale	Uses
World Health Organization Composite International Diagnostic Interview.	A structured diagnostic tool used for assessing mental health disorders in research and clinical settings.
The Maslach Burnout Inventory (Student-Survey) (MBI-SS).	considered the “gold standard” for measuring burnout, encompassing 3 scales: emotional exhaustion, depersonalisation, and personal accomplishment.
The Arabic version of the Geriatric Depression Scale (15 questions).	is commonly used to assess depression in older adults who speak Arabic.
A Patient Health Questionnaire (PHQ-9)	screening tool for depression.

1.2 Antenatal depression

Mood disorders such as depression have adverse social and economic repercussions for individuals and society. Depression has been shown to decrease quality of life and intensify disability (5). Pregnant and lactating women are particularly vulnerable to episodes of depression (17). Research has indicated that 10 to 20% of pregnant women are diagnosed with clinical depression, and more than 25% have depressive symptoms (18–22).

Depression in late pregnancy has been linked with an adverse pregnancy outcome (23), and postnatal depression (24). Postnatal depression adversely influences the child's growth and behaviour due to disruptions in caregiving, attachment, and parent-child interactions, as well as exposure to a stressful environment and potential genetic and biological factors (25). Therefore, preventing antenatal depression benefits the mother’s health and the child’s development.

Maternal depression is defined as depressive symptoms during the antenatal or postpartum periods (20). It comprises minor and major depressive symptoms, and there is no clinical difference between maternal depression and depression at other times (26). Estimated rates of maternal depression vary between 6.5% and 19.8% and are considered a mental health disorder during pregnancy (18–22). Depression affects around one in seven pregnancies, with the prevalence increasing throughout the pregnancy (27).

The depressive symptoms associated with maternal depression are low mood, loss of pleasure, guilt or worthlessness, impaired concentration, sleep disturbance, appetite disturbance, and suicidal ideation (21). These characteristics have negative repercussions both on the mother and the developing fetus. Antenatal depression has been linked with fetal growth restriction and premature birth (27,28), CS delivery, longer hospital stays for newborns (23) and postnatal depression (24). Therefore, the prevention of antenatal depression is likely to be beneficial for both mother and child. While the detrimental effects of maternal depression have been widely acknowledged (29), concerted efforts have been geared toward treatment rather than prevention. Moreover, the failure to effectively treat depressive symptoms is associated with a worse outcome (27,28). Therefore, examining the social or biochemical precipitating factors is essential (30).

Antenatal depression is understood to be multifactorial rather than a result of a particular causal factor (31). It is identified as a complex psychiatric disorder with a multi-dimensional phenotype and includes social/psychological factors and the biological aspects of pregnant women (32,33). The significant risk factors for the onset of antenatal

depression are a previous history of mental health problems, including major depressive episodes or premenstrual dysphoric disorder (34) and poor partner support (35).

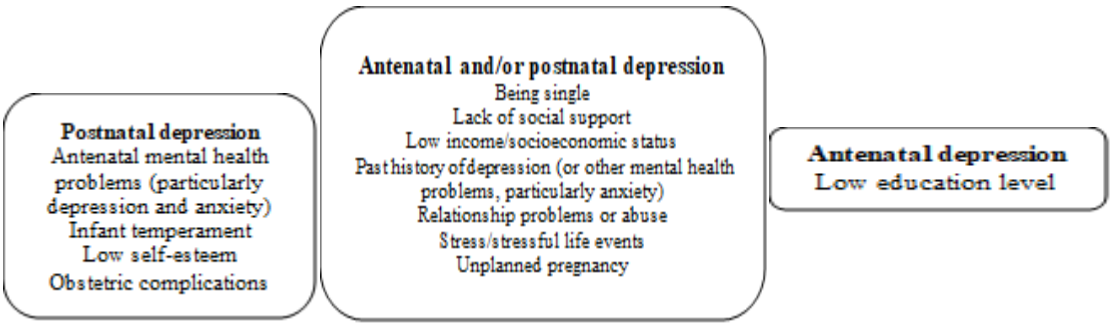

Figure 1-1 The antenatal depression and influencing factors.

1.3 Anxiety and stress in pregnancy

Anxiety and stress commonly affect pregnant women and can significantly impact the mother and child (36). Research on mental health during pregnancy generally focuses on diagnosable psychiatric conditions, mainly anxiety disorders (37,38) and, to a lesser extent, on posttraumatic stress symptoms after difficult experiences at previous childbirth or adverse life events. However, research outside the mental health area offers ample evidence of various clinical symptoms in pregnant women, as assessed with the use of screening tools such as the Edinburgh Postpartum Depression Scale (EPDS) (39).

A large body of research focuses on stress among pregnant women; for instance, In a meta-analysis of more than 100 studies involving 221,974 women, Dennis et al. found that approximately 18.2% of women reported experiencing symptoms of anxiety during the initial trimester of pregnancy (40). Pregnant women are more likely to experience high anxiety symptoms during the first trimester compared to women who are not pregnant. This suggests that the stressors that exist outside of pregnancy may be intensified during

pregnancy or that pregnancy may amplify the manifestation of these stressors during this period. Stressor factors generally affecting pregnant women include pregnancy complications, low family income, heavy responsibilities, unhappy employment status, and pressure in intimate relations (39).

Maternal anxiety is associated with poor neonatal outcomes, including preterm delivery (41,42), low birth weight (LBW) (43), and has adverse effects on infant neurodevelopmental and cognitive outcomes (29). Other adverse consequences of anxiety disorders in pregnant women include unhealthy child development, such as hyperactivity disorder and language delay (44). This is likely due to a combination of physiological stress responses affecting hormonal regulation, disruptions in maternal-fetal signalling, and complex interactions between genetic, environmental, and behavioural factors (36).

1.4 Depression and Anxiety among Pregnant Women in Oman:

In the Arabic world, maternal depression is becoming more recognised, with prevalence rates ranging from 10% - to 37% (45–49). In Gulf countries, where women have a similar culture to that of Omani women, the highest prevalence rate, 37%, is stated in Bahrain (50); the United Arab Emirates has rates between 10% and 20% (51,52); in Kuwait, the rate is 11.7% (53); and in Qatar, the rate is 17.6% (54).

A rising level of pregnancy-related depression has been documented in Oman, both in clinical settings and in the community (55). The authors reported that 12% of pregnant women in Oman had a more significant risk of postpartum depression. Moreover, an observational study involving 959 pregnant Omani women (56) indicates that 24.3% of the study sample suffered from antenatal depression. A recent study among a group of

Omani women found that 27.12% likely had antenatal depression in the third trimester, and 29.30% had postnatal depression at eight weeks after delivery (57). This is considered high, particularly in comparison with findings from most other Arab countries (29,58).

Universal screening for mental health during the perinatal period is not commonly practised in most countries around the globe. However, some countries, including the UK and USA, have established clinical guidelines with different recommendations for screening and assessment of perinatal depression and anxiety (59,60). In Oman, no screening assessment in terms of antenatal care protocols exists to identify women with antenatal depression within the MOH institutions in Oman (61). To date, pharmacological treatment is the primary intervention available to people with depression in Oman. The incorporation of mental healthcare into primary care settings is challenging due to limited resources, such as a lack of interdisciplinary teamwork in most primary healthcare systems in Oman (57). In the UK, the National Institute for Health and Care Excellence (NICE) guidelines state that it is essential to seek alternative treatments for depression before resorting to pharmaceuticals due to the cost-benefit ratio being too high (3) and unwarranted side effects (62).

1.5 Pregnancy outcomes

1.5.1 Preterm birth (PTB)

The WHO states that around 15 million babies worldwide are born prematurely yearly, accounting for around 10% of all births (63). Preterm birth has enormous economic and public health consequences (64). Preterm birth is defined as delivery occurring between the 24th and 37th week of pregnancy (65). It is linked with long-term adverse health and

social outcomes for the mother and her child (66). Babies born prematurely tend to have more complications, including developing jaundice, sepsis, respiratory distress syndrome and even death (63), with long term consequences including developmental delay, vision and hearing issues, and an increased risk of cerebral palsy and other disabilities (67). Moreover, preterm birth can result in enduring consequences for the mother's physical and mental well-being, including an increased risk of postpartum depression and future reproductive problems (68,69).

Preterm birth can be classified as either spontaneous or iatrogenic. Spontaneous preterm labour occurs when the onset of labour and delivery occurs naturally and without any medical intervention before 37 weeks of gestation (70). Spontaneous preterm labour causes are poorly understood, but risk factors include infection, inflammation, multiple pregnancies, and certain medical conditions (71). Latrogenic preterm is medically indicated due to the complications that can occur during pregnancy, such as pre-eclampsia and fetal growth restriction (70).

1.5.2 Fetal growth and birthweight

The weight of infants at birth is an essential aspect that should be considered in clinical practice. The WHO states that about 15% of newborns worldwide have a LBW yearly (72). LBW refers to an infant that weighs less than 2,500 grams (73).

Intrauterine nutrition is one of the significant factors inducing LBW. Other common factors that can also cause LBW include the mother's short stature, preterm birth, low maternal weight, inadequate nutrition, smoking, and the female sex of the newborn (74,75,75–77).

LBW is linked to poor physical and mental development in children after delivery (78). They are prone to adverse health outcomes such as learning difficulties, visual and hearing impairments (79), obesity, and diabetes (80). They can also develop conditions that affect their brain development, such as delays in nerve growth (81). Moreover, LBW babies have a higher chance of neonatal death (82).

Being small for gestational age (SGA) is also known to cause adverse health outcomes and contribute to newborn babies' mortality (65). Around 24% of newborns are born SGA annually (67). At birth, a baby born with a weight that falls below the cut-off of the 10th percentile at gestational age is considered to be SGA. Severe SGA is classified as an infant whose birth-weight falls under the third percentile (83).

SGA is linked with long-term adverse health and social outcomes (66). Smoking during pregnancy, maternal malnutrition, and genetic factors can cause SGA. In addition, babies born with this condition are more prone to experiencing various health issues, such as respiratory distress, hypothermia, and hypoglycaemia. They are also more likely to develop longterm health problems such as diabetes and hypertension (68,69,84).

1.5.3 Gestational diabetes mellitus

Gestational diabetes (GDM) is a type of hyperglycaemia that develops due to an intolerance to glucose and insulin resistance. This is first recognised during the second trimester of pregnancy, whether or not the disorder continues after childbirth (85). It is a common health problem that significantly affects pregnant women and their children (86). GDM is linked with preeclampsia (PE) and CS (87), preterm birth (88), macrosomia, and acute respiratory distress syndrome in the newborn, obesity and T2D (89–91). The

prevalence of GDM worldwide is estimated to vary from 2 to 16% depending on the age group and the diagnostic procedures used to screen it (92).

The GDM risk factors include a history of GDM, maternal age, ethnicity, family history of T2D, and raised BMI. Furthermore, micronutrient deficiency has been linked with GDM onset (93,94). Inflammatory factors during pregnancy are believed to prompt insulin resistance and glucose intolerance among pregnant women. Thus anti-inflammatory mechanisms may help reduce poor GDM outcomes (95,96).

The exact pathogenesis of GDM is unknown; evidence suggests that it can be affected by the disruption of oxidative stress mechanisms and obesity (97). GDM is often linked to a dysfunctional pancreatic B-cell that can lead to T2D after pregnancy (98). In addition, the offspring of women affected by GDM were more prone to developing conditions such as adult attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (99).

1.5.4 Preeclampsia

PE is considered the most common overall pregnancy complication, leading to significant gestational and neonatal morbidity and mortality (100). It affects 6% to 10% of all pregnancies globally (101).

PE is most commonly diagnosed after 20 weeks of pregnancy (102). PE is described as a multisystem disorder unique to pregnant women, generally associated with proteinuria (>300 mg/day), neurological or haematological disruption, and liver and renal complications (103).

In addition to an extended hospital stay in the neonatal special care unit (104), cardiovascular diseases, low birth weight and fetal growth restriction are associated with PE (105).

The exact factors that cause PE are currently unidentified. However, it is believed to be a complex condition that can occur due to improper implantation of the placenta in the early stages of pregnancy (106). Despite this, several theories have been put forward to explain its development. According to the "Modified Two-stage Model", a combination of irregular placentation, vascular abnormalities, and parental factors can contribute to the onset of PE (107).

1.6 Fatty Acids

1.6.1 An overview

Fatty acids (FAs) are the most important groups of lipids. They consist of a carboxylic acid with an extended aliphatic chain of consistently numbered carbon atoms. FAs take three forms cholesterol esters (CE), phospholipids (PL) and triglycerides (TG). For example, phospholipids are critical elements of all cell membranes and are separated into sphingomyelin and phosphoglycerates (108). There are various phosphoglycerates, such as phosphatidyl, phosphatidyl serine, and glycerol. They can also be classified as ethanolamine, inositol, and choline. Phosphoglycerates are usually found in biological membranes (108). Cell membranes are formed by interacting with various lipid components such as cholesterol and glycolipids (109,110). Therefore, red blood cells (RBC), tissues, and plasma membranes contain high levels of FAs, particularly phospholipids (108).

FAs are important for the functioning and growth of body cells (111). Classifying these substances into three groups - saturated, monounsaturated, and polyunsaturated - is determined by their overall carbon atom count. The three varieties of FAs can be distinguished based on the position of the initial dual bond in omega-6, a single bond in omega-3, and a double bond in omega-9 FAs (108).

FAs can be categorised based on their carbon atom count. Those with more than 14 carbon atoms are considered middle and long-chain FAs, while those with less than six carbon atoms are defined as short chain FAs (112). An example of a polyunsaturated (PUFAs) long-chain FAs is linoleic acid (LA), which has a carbon atom count of 18 (113).

In 1930, Burr and Burr coined the phrase "essential FAs" (EFAs) after observing that rats fed with a low-fat diet and suffering from severe skin problems exhibited improved symptoms when fed with small amounts of alpha-Linolenic acid (ALA) and LA (114).

The significance of LA was not understood until Hansen et al, confirmed the association between skin symptoms and growth retardation and milk with a low LA level in human infant (115). Furthermore, the importance of ALA was recognised in 1982 by Holman and his colleagues when they found that insufficient ALA can affect visual impairment and neurological status, and the supplementation of ALA can reduce the adverse effects in human (116).

1.6.2 Synthesis and metabolism of essential fatty acids

The human body contains two primary PUFAs, omega-6 FAs and omega-3 FAs - which are derivatives of two central FAs, LA and ALA (117). These two crucial FAs cannot be produced effectively in the body. Thus, they must be acquired through diet (118). ALA

can be found in walnut and flaxseed, while LA is found in sunflower-seed, soybean, maise, and cottonseed (119). After food digestion in the human body, ALA and LA can convert to stearidonic acid and cis-γ-linolenic acid via an enzyme called Δ6-desaturase (120). The omega-6 parent precursor LA is transformed into arachidonic acid (AA) and dihomo-γlinolenic acid (DGLA). AA is a central membrane component and predecessor for eicosanoids, including leukotrienes and PGs. Eicosanoids are effective cell controllers in regulating inflammation and parturition (118). Leukotrienes, prostaglandins, prostacyclins, and thromboxanes are vital molecules that regulate physiological processes in the body, such as inflammation, vascular tone, uterine contractility, and fetal development, ensuring the body's proper functioning and overall health. AA is vital for central nervous system development, placental function, immune system regulation, and neurotransmission (121).

ALA undertakes a sequence of desaturation and elongation processes, leading to the production of long-chain molecules such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), all of which are omega-3 FAs. However, these processes are limited in the human body, notably in terms of conversion to DHA. Thus, sufficient quantities of DHA and EPA should ideally be provided from nutritional intake, for example, fish or supplements (117). DHA and EPA omega-3 FAs, the two omega-3 FAs that are most critical for human health, are potent anti-inflammatories (120). The conversion action of the Δ6-desaturase is inhibited by ageing, alcohol intake, smoking, a protein-restricted diet, hyperglycemia, high cholesterol level intake, and a shortage of mineral and vitamin co-factors (120,122–128).

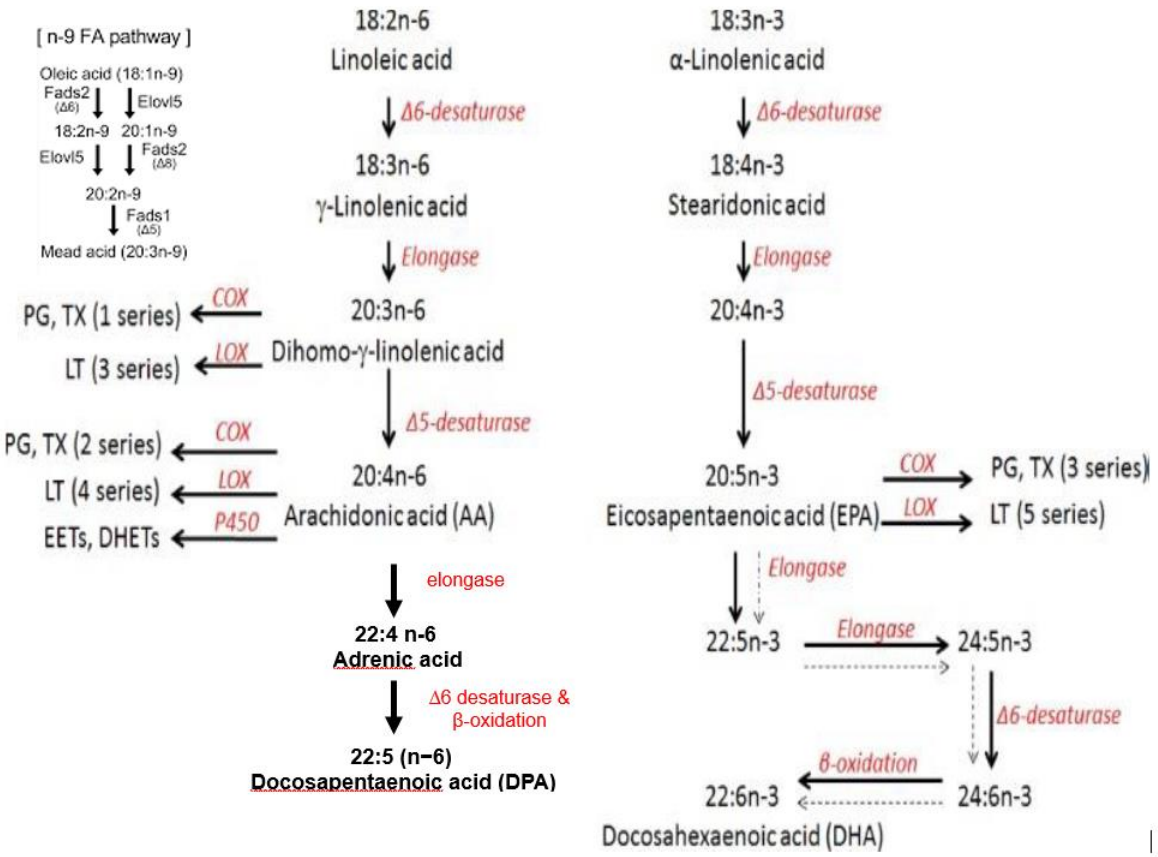

Figure 1-2 Metabolism of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids in the human brain.

1.6.3 Biological marks of essential fatty acid deficiency

Getting enough of the required amount of omega-3 is very important for the body. A deficiency can occur due to insufficient supply. Omega-3 FA deficiency usually arises once less than 2% of the overall energy requirements are obtained from essential FAs (129). However, providing a specific amount of omega-3 fatty acids that account for 2% of overall energy requirements is challenging due to individual variations in energy needs, diverse sources and energy content of omega-3-rich foods, and differences in the body's absorption and utilisation of omega-3 FAs (130). FAs proportions can be utilized to

evaluate the significance of individual FAs in comparison to the overall level of FAs. Fatty acids can alter their relative proportions without changing absolute quantities (131,132).

The biological markers of essential FAs deficiency can occur in a few days in children and about one month in adult patients (129). Dry skin, hair loss, growth impedance and infertility coagulopathies are the most common clinical signs of EFA deficiency (120).

The clinical manifestations associated with ALA and LA are caused by the low level of their lengthier chain metabolites, for example, DHA, EPA and AA. The biological sign of EFA deficiency is the rise in eicosatrienoic acid, commonly called di-homo mead and mead acids (MA). This FAs is transformed by converting oleic acid, a monounsaturated FAs type (133). The transformation of oleic acid to (MA, 20:3 n-9) arises from a low dietary intake of LA and ALA. Therefore, an arachidonic and mead acid ratio lower than 0.3 is associated with LA deficiency (134).

Similarly, a low dietary intake of DHA, with an increase in omega-6 FAs intake, results in a reduction in DHA levels and a rise in DPA created from adrenic acid (ADA). Consequently, DHA/DPA and DPA/ ADA are reliable indicators of the DHA level (135). Recently, the dietary intake in most of Western society has significantly reformed the balance of omega-6 / omega-3 FAs intake from 1:1 to 20-30:1 (136), which could hypothetically stimulate the pathogenesis of numerous chronic conditions (133,137).

1.6.4 Fatty acid deficiency and ill health problems

FAs are essential for cellular functions, gene regulation, and membrane fluidity (138). It is well known that lipids form about 60% of the brain's components; more than 30% of these phospholipids are long-chain FAs, mainly DHA (139). The AA forms about 15% of

phospholipids FAs and exists in all biological membranes (121). For example, a low level of AA during pregnancy and at childbirth is linked with poor pregnancy outcomes such as preterm delivery (64), intrauterine growth delay (140), small head circumference and LBW (141).

Moreover, AA is anti-inflammatory FAs as its the forerunner for creating eicosanoids, mainly lipoxins, thromboxane, and prostaglandins, which maintain endothelial health, normal vascular function and the inflammatory state; a deficiency or imbalance due to AA deficiency may result in excess inflammation or an altered vascular function (121).

Omega-3 FAs EPA and DHA have beneficial effects in many cellular pathways, such as metabolism and the regulation of gene roles (142), both of which might be the cause of many disorders in the human body (143). These include improved visual development and cognitive performance (144,145), strengthened vascular and immune systems (146), and improved visual and neuronal growth (109,147).

Research has demonstrated that a substantial intake of DHA ranging from around 200 to 1000 mg/d through diet can stimulate brain development, particularly during critical periods such as infancy and early childhood (133,147). DHA's unique ability to affect neural cell signalling and its presence in the brain makes it an ideal candidate for developing symbolism and self-awareness (148). Moreover, patients with DHA supplements for peroxisomal disorders have enhanced social contact, improved liver function, increased myelination and muscle tone, and improved vision (149).

The beneficial role of omega-3 FAs supplements on cardiac diseases was earliest underlined by (150). Since then, several studies have indicated the valuable role of DHA

and EPA supplementation on stroke and fatal CHD (151,152). The cardio-protective role of EPA and DHA supplements is due to their influence on endothelial and vascular functions and their anti-aggregatory action due to the impact on clotting to prevent atherosclerosis. Furthermore, they have beneficial effects on inflammatory bowel disease (153), arthritis (154), atopic diseases (155) and prostate, breast, and colon cancer (18).

1.6.5 Dietary recommendations with regard to Omega-3 fatty acids

Pregnant women receive a wide variety of public health advice regarding seafood and Omega-3 FAs intake. In the USA, the American Heart Association (AHA) advises increasing the dietary intake of foods and oils rich in ALA or eating oily fish twice weekly for healthy adults (146). Meanwhile, the 2020-2025 Dietary Guidelines for Americans state that women must eat 8 to 12 ounces of fish (227–340 g) per week and 375 mg/day of omega-3 FAs during pregnancy (156). Furthermore, the Food and Drug Administration (FDA) recommends more than 3 g/day of omega-3 FAs, of which a maximum of 2 g/day should come from fish oil supplements (157). In Europe, the European Food Safety Authority (EFSA) recommends 2g of ALA FA and no more than 250 mg of DHA and EPA daily (158). Moreover, in 2014, the same agency noted that eating around 3 to 4 portions of fish per week (>450g/week) could provide neurodevelopment-related nutritional benefits compared with no fish intake (159), Furthermore, in the United Kingdom UK, the government’s recommendation for pregnant women is to consume about 450 mg of DHA and EPA daily, or to eat two meals of fish weekly (140 gm = one portion), one of which must be in the form of oily fish (160).

The FAO/WHO joint expert consultation 2008-2010 on Fats and FAs in Human Nutrition and the International Society for the Study of FAs and Lipids (ISSFAL) advise pregnant

women and nursing mothers to consume, as a minimum, 0.2 g/d DHA or 0.3 g/d EPA+DHA from dietary sources (161).

The Omani Guide to Healthy Eating suggests that healthy adults consume around five servings of fish per week, with one serving equivalent to 30 g. Nonetheless, no recommendations have been made regarding fish consumption during pregnancy (162).

Evidence shows that fresh tuna, mackerel, sardines, pilchards, anchovies, and salmon are a direct food supply of omega-3 FAs (163). This is because the omega-3 FAs in oily fish are more efficiently integrated inside plasma phospholipids than if intake is in the form of a nutritional supplement (164).

Table 1-2 Nutritional requirements during pregnancy:

Macronutrient	EAR for energy	Micronutrient	Recommended Intake
Total Energy (kcals)	2100 kcal - 2200 kcals	Iron	(14.8 mg)
Protein %EI	15% of EI	Calcium	(700 mg)
CHO %EI	50% of EI	Iodine	(140 µg)
Total Fat %EI	35% of EI	Vitamin D	(10 µg)
SFA %EI	11% of EI	Folate	(300 µg)
MUFA %EI	13% of EI	Vitamin A	(100+ µg)
PUFA %EI	13% of EI	EPA + DHA	(450 mg)

DRV—Dietary Reference Values; EAR—Estimated Average Requirements; EI—Energy intake;. CHO-carbohydrate; Saturated fatty acid; MUFA—Mono-unsaturated Fatty Acids; PUFA— Polyunsaturated Fatty Acids; polyunsaturated fatty acid:

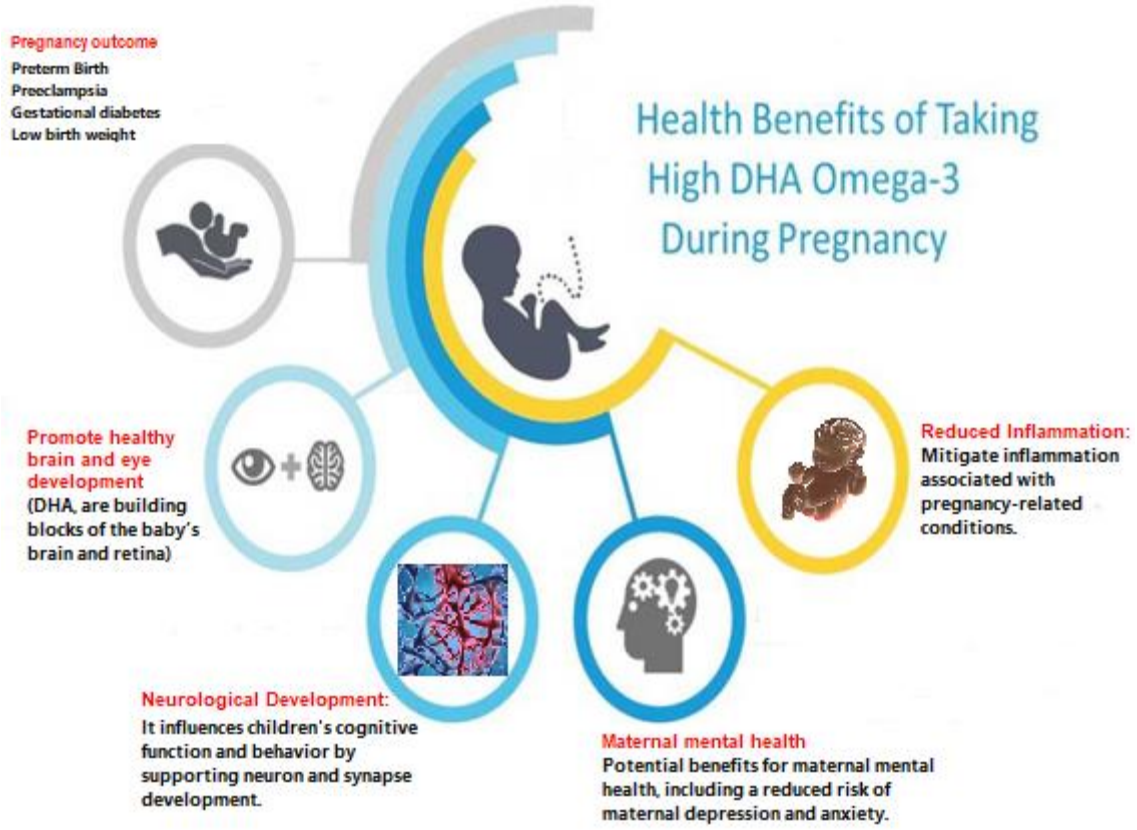

Figure 1-3 Omega-3 Fatty acids' beneficial effects during pregnancy.

1.6.6 Fish and seafood intake during pregnancy

According to various studies, fish consumption in pregnant women is often below that recommended in many countries worldwide (165). This is a concern as fish is an essential source of nutrients such as vitamins, protein, and omega-3 FAs, all of which are essential for the development of the fetus (121). Fish is rich in nutrients such as zinc, magnesium, and selenium, which have been associated with alleviating symptoms of anxiety and depression. Zinc regulates the brain's response to stress, magnesium aids in relaxation and stress reduction, while selenium is crucial for antioxidant function and reducing the

risk of depression (166). Fish is a high-quality source of complete protein, containing all essential amino acids for maintaining brain health and synthesising neurotransmitters (167). Tryptophan, present in fish, acts as a precursor to serotonin, thereby contributing to feelings of well-being and happiness (168). However, fish contain traces of methylmercury (MeHg), and public health nutritional guidelines on seafood intake have been developed to address the main objective of limiting people's exposure to this potentially harmful substance (169). Consequently, pregnant and breastfeeding women are advised to limit oily fish intake to no more than 1–2 meals a week and avoid consuming some fish, including marlin, swordfish, and shark (170,171). These guidelines are constructed on a cautionary approach and recommend that people avoid eating fish with high MeHg content (169).

Fish consumption throughout pregnancy is more likely to meet the nutritional requirements of these primary nutrients (172). Extensive evidence supports the nutritional value of eating fish during the prenatal period (173–175). The current recommendations about fish intake for pregnant and breastfeeding women are to limit oily fish intake to no more than 1–2 meals a week and avoid consuming some fish, including marlin, swordfish, and shark (170,171). However, there is growing evidence that high seafood and fish intake during pregnancy is beneficial for the fetus's brain and for child neurodevelopment (176–178), probably because of the range of essential nutrients contained in fish for example, omega-6 and omega-3 FAs, vitamins B12 and D, iodine, iron, selenium and zinc (174,179). Moderate mercury levels, B-Hg level (+32.9%) particularly in oily fish, do not seem to have adverse effects (178).

Table 1-2 Fish intake recommendations of mercury content during pregnancy:

Best Choices **EAT 2 TO 3 SERVINGS A WEEK**	**Good Choices** **EAT 1 SERVING A WEEK**	**Choices to Avoid** **HIGHEST MERCURY LEVELS**
Anchovy, Haddock, Salmon Whiting, Hake, Sardine, Herring Scallop, Atlantic croaker, Lobster, American and spiny, Shad, Atlantic mackerel, Shrimp, Skate, Mullet, Black Sea bass, Snapper, Smelt, Oyster, Butterfish Pacific chub mackerel, Sole, Catfish, Squid, Clam, Perch, freshwater and ocean, Tilapia, Cod, Trout, freshwater, Crab, Pickerel, Tuna, canned light (includes skipjack), Crawfish, Plaice, Flounder, Pollock, Whitefish	Bluefish, White croaker, Pacific croaker, Monkfish, Buffalo fish, Rockfish Tuna, albacore/ white tuna, canned and fresh/frozen, Tuna, yellowfin Weakfish/ seatrout, Carp, Sablefish, Chilean sea bass/ Patagonian, Sheepshead, Grouper Toothfish, Halibut, Mahi Mahi/, dolphinfish, Spanish mackerel, Striped bass (ocean), Tilefish (Atlantic, Ocean)	King mackerel Shark Tilefish (Gulf of Mexico) Marlin Swordfish Tuna, bigeye Orange roughy
The FDA's safety limit for mercury in fish is 1 part per million (ppm) (170,171).		

In the Avon Longitudinal Study of Parents and Children (ALSPAC), which analysed over 14,000 pregnancies, mercury levels were measured in the blood and in terms of fish intake during pregnancy, with a follow-up of the children involved to 8 years of age (177). It was found that mothers who ate more than three portions of seafood per week saw positive effects on child brain development (180).

In contrast, women with no or low fish and seafood intake during pregnancy had the worst outcomes in terms of behavioural scores, verbal intelligence quotient (IQ) and motor communication in children at eight years of age (177). Moreover, further investigation of the ALSPAC data shows that limiting fish intake in pregnancies had a negligible effect on blood mercury levels (176). Nor was the level of mercury in the blood of pregnant women

related to either preterm birth or birthweight (178), heart rate or blood pressure in children up to age 17 (181), or on the child development (179). These findings suggest that the recommendation to limit fish consumption among pregnant women is harmful (174,177).

Numerous studies show that the population with higher seafood intake during pregnancy have little to no risk of adverse effects on the neurocognitive development in newborn and young children (182–185). For example, the Japanese population consumes more seafood than any other nation (186). Consequently, pregnant women in Japan have a relatively high level of mercury in the blood (187), but these levels are not associated with small gestational age or low birth weight (188–190). Furthermore, several international organisations have stated that oily fish intake with low methylmercury content is linked with healthier neurodevelopmental outcomes in children. The advice ranges between 2 to 4 fish portions per week (191–193).

1.7 Omega-3 Fatty Acids and Depression in the general population

Oily fish is the primary nutritional supply of omega- 3 FAs, vital for several metabolic pathways, and have varied effects on several physiological and biochemical functions (138). Epidemiology studies have highlighted that societies with higher fish consumption have a lower rate of mood disorders, including depression. Conversely, those populations with low fish consumption have been associated with a greater rate of mood disorders (194,195), as well as other parameters directly affecting health (196).

Omega-3 FAs are recommended to adjunct therapy for people with psychotic disorders and depression due to their essential role in brain development and regulating neurotransmitter activity, including the action of serotonin and dopamine, which are necessary neurotransmitters that affect mood, behaviour, and cognition (112).

To evaluate the beneficial role of omega-3 FAs on mental illness, it is essential to understand the neurobiological changes that appear during a depressive episode, although the exact pathogenesis remains unclear (197).

Omega-3 FAs stimulate many neurobiological processes which seem to play a part in depressive disorders (198). Omega-3 FAs alter the membrane lipids' role and affect the usual transmitter signalling in the cell, which might influence the pathophysiology of depression (198).

Omega-3 FAs aid neuronal transmission by creating flexible cell membranes. It has been suggested that the difference in FAs levels alters membrane fluidity, resulting in unbalanced neurotransmitter systems. The pathophysiological activities of some of the neurotransmission systems have been critically linked to depression; conversely, the modulation of these neurotransmitter systems has reduced symptoms of depression (195). In addition, omega-3 FAs alter cellular immune responses in the body (199). Furthermore, a high level of DHA in the brain has important physiological roles, including dopaminergic and serotonergic transmission, cellular signal transduction and regulation of cell membrane fluidity (200–202). A lack of omega-3 FAs is linked with various pathological conditions, including antioxidant system deficiency (203). Omega-3 fatty acids and antioxidants collaborate to boost the body's resilience against oxidative stress. For example, EPA and DHA exhibit anti-inflammatory properties, which not only boost the effectiveness of antioxidant enzymes but also directly combat free radicals (204) This partnership fortifies the body's antioxidant defences, diminishing oxidative harm and fostering general well-being. Many studies have indicated that major depressive disorder is an inflammatory state linked with high pro-inflammatory cytokines (200,201). Omega-3

is found in cell membranes, especially the central nervous system, as it is integrated into cell membranes to structure the lipid bilayer and trigger neuronal apoptosis (205). Furthermore, T-cells act on neuroprotective and anti-inflammatory functions through inflammation by modulating immune repression. They help maintain immune balance and protect against neuroinflammatory conditions. This implies that impaired T-cells might directly affect the onset of depression (206,207).

1.7.1 Epidemiology and prevalence studies

Numerous systematic reviews show a significant relationship between seafood and omega-3 FAs intake and levels and prevalence of depression. A meta-analysis involving 20,000 patients with depression from 31 observational studies was used to assess the positive effects of high fish intake and omega-3 FAs on mental disorders, including depression (208). This study linked low fish intake with high rates of depression. Moreover, a meta-analysis including more than 6500 individuals with depression from 10 observational studies found a moderate association between omega-3 FAs intake with depression symptoms, mainly in the case of women (209). Clinical trials frequently confirm a close relationship (210,211). What is more, patients diagnosed with major depression have low omega-3 FAs status compared to the control patients (212), Low omega-3 fatty acid levels in patients with major depression may be both a cause and an effect of the condition. Low omega-3 levels may raise depressive symptoms due to the critical role of these fatty acids in brain health and mood regulation. Low EPA and DHA omega-3 FAs levels in erythrocytes (213), and low omega-3/omega-6 FAs ratio, specifically EPA/AA (214). The lower levels of DHA and total omega-3 FAs were linked with higher anxiety and depressive symptoms (215) and postpartum depression (117).

Consistent with these results, patients with mood disorders had lower omega-3 FAs in blood samples (216). Likewise, a longitudinal study of a younger age group (18–30 years) reported, after a 3-year follow-up, that a low risk of depressive symptoms was linked with a high omega-3 FAs intake (217).

Regarding the elderly population, (218) reported that low fish intake was linked with moderate depressive symptoms. However, the study included a group of elderly Japanese people with depression and showed no difference in the FAs profile between the control and the case samples (219).

These outcomes do not necessarily indicate a causal relationship between low oily fish and omega-3 FAs intake and a higher rate of depression symptoms. Several economic, social, cultural, and other factors can muddle this association (220), as many studies have been unsuccessful in reporting a significant relationship linking omega-3 FAs intake and depression symptoms or only indicated a weak correlation that is entirely attributable to confounders (221,222). At the same time, some studies indicate no relationship between omega-3 FAs levels in erythrocytes and depression symptoms (223,224). This was substantiated in longitudinal studies among older people (50–69 years) after an 8-year follow-up (222). Moreover, studies dealing with different ethnic and age groups found no association linking omega-3 FAs intake and levels and the prevalence of depression (225,226). Review studies demonstrated a significant inconsistency between studies but verified the positive role of omega-3 FAs intake in reducing depression symptoms (227,228). This implies that further studies are warranted in subjects with a pre-defined fatty acid profile.

1.7.2 Clinical and intervention studies

In a clinical trial, 28 depressive patients were randomly selected to take 3.3 g/day of a mixture of DHA and EPA for two months (229). The researchers reported that participants in the FAs treatment group had a significant reduction in their Hamilton Depression Rating Scale (HDRS-SF) score ($P<0.001$). Conversely, in clinical trials, 37 depressed participants who took 2 g/day DHA for 1.5 months did not report any beneficial effects (230). This outcome was replicated in other controlled clinical trials with similar aims using DHA supplements for the treatment group (231).

Clinical trials have shown conflicting results regarding the application of DHA supplementation to mitigate symptoms of depression to treat depression. Some claim that it is not DHA but EPA (222,231–236) that has an effect. However, Peet et al, (237) found that high doses of EPA (4 g/day and above) have little bearing on the symptoms of depression, while a low dose of 1 g/day of EPA has the most effect on symptoms. Peet reported an improvement in HDRS-SF score when using an EPA supplementation of 1 g/day or 2 g/day in a 3-month clinical trial (n= 75) on patients with bipolar depression. However, there was no significant trial or significant effects in another clinical trial using 6g/day of EPA as an adjunctive therapy (238). Moreover, Sinclair et al, (239) found a beneficial effect with low doses of a mixture of DHA and EPA (3.5 gm) or EPA alone (1-2 g/day). However, DHA alone (2 g/day) had no significant effect, nor did EPA alone (4-6 g/day). Therefore, more studies dealing with dosage issues and ones using brain-specific supplements are imperative.

Furthermore, in a randomised controlled trial (RCTs) with regard to omega-3 supplementation effects on decreased serum cytokine production and depressive

symptoms in individuals who were sedentary and overweight, the data suggested that omega-3 FAs supplements have beneficial effects on inflammation (240). In a 4-month trial, the investigators assessed different doses of ω-3, EPA and DHA supplementation. The researchers used 2.5 g/d Omega-3 of a 7:1 EPA/DHA ratio and demonstrated that EPA had a comparatively more substantial antidepressant and anti-inflammatory effect than DHA. It became clear that a significant alteration in cytokine production by lymphocytes only arises with more than 2.0 g/d of EPA and DHA (241). Martins et al, reported better omega-3 FAs effects with a shorter intervention time in a more homogeneous cohort. In a RCTs, (242) indicated that 2.5 g/day for two months significantly improves depressive symptoms in elderly patients. Furthermore, RCTs aimed to estimate the efficacy in treating Major Depressive Disorder (MDD), using 2 g/d in a 5:1 ratio of EPA: DHA for eight weeks. The authors found a decrease in symptoms of MDD in the treatment group (243). Short term supplementation did not detect a significant effect (231).

There is insufficient data from the literature on the optimal doses and ratios of DHA and EPA omega-3 FAs supplements for the management of depression symptoms. In addition, a Cochrane review published in 2021 included around 1,400 participants and analysed the results of RCTs that examined the efficacy of omega-3 FAs supplements in reducing major depressive symptoms. They found a small to moderate reduction in depressive symptoms compared to a placebo (244). They found that the daily ratio and the dose of both DHA and EPA of which (EPA > 50%, 60%, and 80%) significantly affected the effectiveness of omega-3 FAs supplements for treating depressive symptoms (245).

1.8 Omega-3 fatty acids and depression and anxiety symptoms during pregnancy

In developed countries, studies have indicated that pregnant women typically benefit from high nutritional requirements but do not always meet high micronutrient needs (169,172–175,246). During pregnancy, the dietary requirements for fetal development and the metabolic and physiological needs of the mother are extensive (247). In the late stage of pregnancy, the fetal requirement with regard to DHA is estimated to be about 50–70 mg/day to ensure fetal brain growth (248). This is important because super-unsaturated FAs are regulated and selectively shifted to the fetus through the placenta (110,249) and incorporated into the central nervous system (250) and the photoreceptors of the retina (251).

DHA is a significant element of all cell membranes and is combined in great concentrations in the membrane phospholipids of the retina and brain (26). DHA comes from maternal stores or dietary intake with little input from the oestrogen-stimulated conversion of alpha-linolenic acid (ALA) to DHA (252). Fetal development requires arachidonic acid (AA) from omega-6 FAs and DHA (1:1); these FAs are critical structural building blocks of the fetal brain (121). Consequently, some pregnant women will be at significant risk of omega-3 deficiency and will have no or tiny amounts of DHA left over to meet their biological needs (249). Consistent with this, maternal plasma DHA levels gradually decline, even with a balanced diet (253). Furthermore, DHA levels can decrease to 50% of the necessary amount during pregnancy and only return to normal at six months postpartum (254). The depletion of maternal omega-3 FAs might increase the incidence of antenatal depression (255–257). Moreover, maternal diet, specifically preformed DHA,

affects the accumulation of DHA in the brain (258). However, Cosatto et al, propose that the maternal brain releases DHA to meet the fetal's needs, which could contribute to antenatal depression development, but more research is needed (259). Current observational findings and RCTs have examined the potential effects of omega-3 FAs intake and level on antenatal depression (260). However, the exact association of this association has remained elusive (117).

1.8.1 Epidemiology and prevalence studies

1.8.1.1 Association between dietary intake of omega-3 fatty acids and prenatal depression

A summary of related observational studies that examine the relationship between fish or omega-3 FAs intakes and pre-and postnatal depression is shown in Table 1-1. Three longitudinal cohort studies dealing with fish and omega-3 FAs intake show a significant protective outcome for the prevalence of antenatal depression. They suggest that a low intake of omega-3 FAs, such as DHA, is linked to higher depressive symptoms in pregnancy (260–262).

In comparison, others report that a high omega-6/omega-3 FAs ratio of ≥9:1 during early pregnancy is linked with a higher incidence of depressive symptoms (263). Another four investigates found no relationship between omega-3 consumption and depression in pregnant women (259,264–266).

Table 1-3 Association between dietary intake of omega-3 fatty acids and prenatal depression:

Study Author Location	Quality [a]	Study Design	Sample size N Target Study group Participants/ Inclusion criteria	Measures Methods	Major finding Results	Author's critical appraisal
(Álvarez-Ramíre et al 2018) Mexico	acceptable	cross-sectional and analytic	151 pregnant women 2nd trimester of pregnancy	(EPDS) (≥12) FFQ (STAI) > 40	a negative correlation between low dietary intake of EPA and DHA, anxiety symptoms (AS) and depressive symptoms (DS) in the 2nd trimester of pregnancy.	Two mental assessment scale and two assessment points but low sample size.
(Golding et al. 2009) UK	acceptable	Longitudinal	14,541 pregnant women 3rd trimester of pregnancy	FFQ EPDS (≥13)	There is an association between low omega-3 intake from seafood and an increased risk of high levels of depressive symptoms during the last trimester of pregnancy.	Larg sample size. No assessment for anxiety. Secondary analysis of data.
(Hamazakia et al 2018) Japan	acceptable	Longitudinal	104,102 pregnant women early, mid and late pregnancy	FFQ EPDS (≥9)	fish intake was associated with a low level of depressive symptoms. However, the associations were weaker for n-3 PUFA intake than for fish intake.	Very large sample. No assessment for anxiety. Low cut off point of EPDS scale
(Miyake et al. 2013) Japan	acceptable	cross-sectional	1745 pregnant women early - late pregnancy	(CES-D scale) (≥16): Dietary history questionnaire	High intakes of fish, EPA, and or DHA were associated with a low risk of depressive symptoms during pregnancy.	Large sample size. No potential confounders data. No generalizability of the findings

(Sontrop et al. 2008) Canada	acceptable	cross-sectional	2394 pregnant women 2nd trimester of pregnancy	FFQ EPDS (≥16)	No association between intake of EPA+DHA and depressive symptoms. The association was only observed among current smokers and women of single/separated/divorced marital status	Large sample size. Good reflection of potential confounders. No association.
Cosatto et al. (2010) Australia	low	Cross-sectional	94 pregnant women 2nd trimester of pregnancy	FFQ EPDS (≥10)	- pregnant women with EPDS (≥ 10) are at risk of developing postpartum depression - low n3-PUFA intakes in pregnant women -No association between n3-PUFA intakes and developing postpartum depression	Small sample size. No assessment for anxiety. No prenatal depression assessment. Low cut off point for EPDS.
da Rocha and Kac (2012) Brazil	low	Prospective cohort	106 Pregnant women in the first trimester of pregnancy	FFQ EPDS ≥ 11	ω6/ω3 ratio ≥9:1 was associated with postpartum depression	Low sample size. No consideration for prenatal depression. Need further analysis.
Strom et al. (2009) Denmark	Low	Longitudinal	(54,202) Pregnant women 2nd trimester of pregnancy	FFQ History of depression, admitted to hospital for depression,	No link between intake of ω3-PUFA intakes or fish and PPD postpartum depression	Large sample size, but no depression screening only hospital visits.
[An] Assessment of Methodological Quality using the CASP checklists for case-control and cohort studies and the (NOKC) checklist for cross-sectional studies. Acceptable in quality for the Studies that score above 50% and studies that score lower or equal to 50% are low in quality.						

1.8.1.2 Association between low levels of omega-3 fatty acids and pre- and postnatal depression

Table 1 2 summarises observational findings examining the association between erythrocyte, plasma, breast milk, and pre-and postnatal depression. Other studies have assessed this relationship by measuring the erythrocyte membrane and serum or plasma omega-3 FAs levels and related them to depressive symptoms in pregnant women; low DHA concentrations or upper n-6/n-3 ratios correspond to a high rate of antenatal and postpartum depression (256,263,267–273). However, similar studies have yet to replicate these findings (274,275).

Table 1-4 Association between maternal omega-3 FAs levels in erythrocyte, plasma, breast milk and pre- and postpartum depression:

Study Author Location	Quality [a]	Study Design	Sample size N Target Study group Participants/ Inclusion criteria	Measures Methods	Major finding Results	Author's critical appraisal
(Changa et al, 2018) Taiwan	Acceptable	case-control	pregnant women (17 PND cases and 16 healthy controls) 2nd trimester or 3rd trimester of pregnancy.	RBC fatty acid analysis (DSM-IV) (EPDS) (≥12)	Prenatal depression is significantly associated with lower DHA, EPA, and total n-3 PUFAs levels and an increased n6/n-3 PUFAs ratio) in the 2nd trimester and 3rd trimester of pregnancy.	Low sample size No dietary assessment. Two assessment points. Need to confirm the association in larger sample size.
Parker et al. (2015) Australia	Acceptable	cross-sectional	900 pregnant women during the third trimester of pregnancy	RBC PL fatty acid analysis EPDS (≥10)	Lower ω3, EPA level and higher n6/n3 ratio were significantly associated with postpartum depression.	Large sample size. Low cut off pint for EPDS. Need dietary assessment.
(Keim et al 2012) USA	Low	Longitudinal	287 pregnant women 2nd trimester of pregnancy	(CES-D scale) (≥16): Breast milk LC-PUFA content (4 months postpartum)	depressive symptoms were inversely associated with breast milk DHA and total ω3 within at 2nd trimester of pregnancy.	FAs levels were only assessed in breast milk. No FAs assessment in blood or diet. Low sample size
(Pinto et al. 2017) Brazil	Acceptable	Cohort	172 pregnant women	EPDS score ≥11	Low levels of DHA, EPA and DPA and a higher n-6/n-3 ratio	low sample size. Low cut off point for EPDS.

			early - late pregnancy	Serum fatty acid analysis	were associated with increased depression rate during pregnancy	No assessment for anxiety. Need diet assessment.
(Hoge et al 2019) Belgium	Acceptable	Cohort	72 pregnant women Early - late pregnancy	RBC fatty acid Bromley postnatal depression questionnaire	Low n-3 PUFA status and high n-6 / n-3 ratio in early pregnancy were associated with postpartum depression.	Low sample size No EPDS. No diet or prenatal depression assessment.
(Rees et al. 2009) Australia	Acceptable	Case-control	16 depressed and 22 non-depressed pregnant women 3rd trimester of pregnancy	plasma fatty acid analysis (DSM-IV) (EPDS) (≥13)	High plasma levels of DHA, total n-3, and a low n-6/n-3 ratio were associated with lower odds of depression.	Low sample size No RBCs FAs. No diet assessment. Need to increase sample size to confirm the association.
Chong et al. (2015) Singapore	Low	Longitudinal	962 pregnant women 2nd trimester of pregnancy	Plasma fatty acid PL analysis EPDS (≥13)	No association between plasma omega-3 levels and pregnancy prenatal and postpartum depression.	Large sample size. No RBCs FAs. No association.
Markhus et al. (2013) Norway	Acceptable	prospective cohort	43 pregnant women 3rd trimester of pregnancy	RBC fatty acid -EPDS (≥10)	The low omega-3 level was associated with higher postpartum depression.	Low sample size No diet or prenatal depression assessment. Low cut off point for EPDS.
(Sallis et al 2014) UK	Low	Longitudinal	5222 pregnant women 3rd trimester of pregnancy	RBC fatty acid analysis (EPDS) (≥12)	No association between levels of total Omega-3 or EPA or	Large sample size. No dietary assessment. No association.

					DHA and perinatal onset depression	
(Shiraishi et al 2015) Japan	Acceptable	cross-sectional	329 Pregnant women 2nd trimester of pregnancy	plasma fatty acid analysis (EPDS) (≥8) FFQ	-Lower plasma DHA concentration was significantly associated with prenatal depressive symptoms - Dietary intake of n-3 was not associated with depressive symptoms	Low sample size. Good to include plasma and diet assessment for FAs. Low cut off ppint for EPDS.
[An] Assessment of Methodological Quality using the CASP checklists for case-control and cohort studies and the (NOKC) checklist for cross-sectional studies. Acceptable quality for studies that score above 50% and s, studies that score lower than or equal to 50% are low in quality.						

1.8.2 Clinical and intervention studies

1.8.2.1 Clinical trials of omega-3 fatty acid supplementation and pre-and postnatal depression

A summary of the RCTs examining the efficiency of omega-3 FAs supplements in reducing pre- and postnatal depression symptoms is shown Table 1 3. Several clinical controlled trials (276–279) have shown that a dosage of EPA > DHA ratios (1.9 g per d: 3.5 g per day for eight weeks can decrease depression symptoms in the postnatal period if given to women in the mid-term of pregnancy, which representative erythrocyte levels of (EPA 3.8 and DHA:5.4%) after 8 weeks of intervention. This outcome was replicated in a RCTs (280) using a daily dose of DHA (300 mg/d) for about 20 weeks in women in the third trimester of pregnancy. Moreover, two clinical trials have shown positive effects using a mixture of DHA and EPA (ranging from 1.3 to 1.5 g/day) for 4 to 8 weeks in women diagnosed with prenatal depression (277,281,282). In contrast, five RCTs using a similar design and an intervention time of 8 to 31 weeks, with a daily dose of a mixture of DHA and EPA (1 g/D to 4.37 g/D), did not show potential benefits in terms of depression symptoms among women postpartum (255,283,284), or women with previous medically-diagnosed depression (285). This implies that supplements are good at preventing depression in high-risk women but not at treating depression when established and that studies dealing with low-risk women have to involve large numbers of such women (286). Furthermore, two clinical trials measuring omega-3 FAs supplements over a long intervention time (6 to 16 months) during pregnancy and after delivery failed to report a significant reduction in depressive symptoms. They used a mixture of DHA and EPA supplements (2.05 g/day) for women diagnosed with prenatal depression (287) and DHA and EPA supplements (1.33 g/day) for women with a history of perinatal depression (288).

The effective doses of omega-3 supplements for anti-depressive effects in postnatal women remain unclear. For example, researchers failed to note a significant reduction in depressive symptoms among breastfeeding mothers when using 0.2 g/d of DHA supplement for four months. The outcome of the (289) trials could be justified by the use of a lower DHA dose than the required dose (0.3 g/d) for lactating women as recommended by several groups to completely replace DHA in the brain (290,291). Furthermore, (Judge et al., 280) showed a significant prevention effect in women with postnatal depression when using supplementation dose as DHA recommendations for pregnant women (DHA of 0.3 g/d). In contrast, a large double-blind RCTs that included 2,399 pregnant women found that 0.8 g/d of pure DHA supplementation was not beneficial (285). This lack of efficacy might be attributed to factors such as dose-response relationships, individual differences in response, and limitations in the study's design.

Some clinical trials examining the potential benefits of omega-3 FAs on antenatal and postnatal depression did not indicate significant effects when using supplements with a low ratio of EPA to DHA (283,285–288) or pure DHA supplement (292). Alternatively, clinical trials using pure EPA or a high EPA to DHA ratio supplement (1 to 3.5 g per d) for approximately two months showed a significant reduction in depressive symptoms among pregnant women (276–279) and postpartum depression (281,282).

Table 1-5 Efficacy of omega-3 FAs supplementation on reducing pre- and postnatal depression symptoms:

Study Author Location	Quality [a]	Sample size N Target Study group Participants/ Inclusion criteria	Dose (g/d) (Omega-3 or fish oil)	Intervention time	Major finding Results	Author's critical appraisal
Freeman et al. (2006a) USA	low	15 Pregnant women with major depressive episodes participated. e ≥16 weeks gestation.	2.8 g (1.7 of EPA and 1.1 g of DHA).	2–14w postpartum	− 1.375 −8.3 - EPDS score: ↓40.9% -HRSD score: ↓34.1% Null	Open trail. Low sample size. Low intervention time. DHA < EPA
Su et al. (2008) Taiwan	Acceptable	33 Pregnant women with MDD	DHA 1200 mg + EPA 2200 mg vs. placebo	(16–32 week gestation)	−1.833 −8 -HAM-D, EPDS: DHA lowered both. depression scores Positive	Low sample size. Low intervention time. DHA < EPA. 3 mental assessment scale.
Judge et al. (2014) USA	Acceptable	42 Pregnant Women	300 mg DHA and placebo	24 to 40 weeks gestation Assessment from 2 weeks to 6 months postpartum.	20 -PDSS scores (2 w,6 w,3 m): significantly lower in DHA group, the DHA group had fewer. PPD	Low sample size. DHA only. Longer intervention time. High efficacy with no EPA. No prenatal depression screening.

Freeman et al. (2008) USA	Acceptable	59 women with erinatal MDD	DHA 800 mg + EPA 1100 mg +psychotherapy vs. Placebo + psychotherapy	Between 12w-32w gestation for 8 weeks	−1.375 −8 -EPDS (pre, post): Placebo (15.83, 8.09) ; EPA/DHA (17.11, 10.96) -HAM-D (pre, post): Placebo (17.43, 9.91) EPA/DHA (18.86, 12.82) -No significant difference Null	Low sample size. Low intervention time. No EPDS psychotherapy is included in either group. DHA < EPA. No efficacy.
Mattes et al. (2009) Australia	Acceptable	83 Healthy pregnant women (21–40-week gestation)	Fish oil 4000 mg (56% DHA, 27.7% EPA) vs placebo	20 w gestation to delivery	−0.125 −19 - EPDS scores (> 12): 9.67% vs 11.19% (DHA vs control) -No significant difference Null	Low sample size. DHA > EPA. longer intervention time. No efficacy.
Makrides et al. (2010) Australia	Acceptable	2399 healthy women	DHA 800 mg + EPA 100 mg vs. placebo	< 20w gestation to delivery	Null	Large sample size. DHA > EPA. 8:1. Longer intervention time. No efficacy.
Marangell et al. (2004) USA	Acceptable	7 Pregnant women with history of PPD	2.96 EPA + DHA −1.4 −17	began at 34th ∼36th week until 12 weeks postpartum	No effect on PPD	Very low sample size. No efficacy.

Mozurkewich et al. (2013) USA	Acceptable	126 pregnant women with EPDS > 9 or a history of depressive symptoms	DHA 274 mg+ EPA 1060 mg vs. DHA 900 mg + EPA 180 mg vs. placebo	From 12–20 weeks gestation to 6–8 weeks postpartum	- No significant associations between serum EPA, DHA and BDI scores Null	Smale sample size. 3 arms trial. 3 different doses. longer intervention time. No efficacy.
Rees et al. (2008) Australia	Acceptable	26 pregnant women with MDD	Fish oil 6 g (27.3% DHA, 6.9% EPA) vs placebo	Between the third trimester and 6 m postpartum for 6 weeks	-EPDS (DHA/EPA vs Placebo) 17.3 vs 16.5 (0 wk) 8.5 vs 9.0 (6 wk) -HDRS (DHA/EPA vs Placebo) 19.7 vs 9.0 (0 wk) 7.9 vs 0.7 (6 wk) -MADRS (DHA/EPA vs Placebo): 30.2 vs 29.2 (0 wk) 13.5 vs 15.1 (6 wk) -No significance between Groups Null	Smale sample size. DHA > EPA. longer intervention time. Two assessment scale. No efficacy.
Doornbos et al. (2009)	Acceptable	119 Pregnant women (14–20 week gestation)	DHA 220 mg OR DHA 220 mg + AA 220 mg vs. placebo	16w estuation to 3 m postpartum	RBC DHA levels (placebo, DHA, DHA + AA): 4.44%, 5.51%, 5.57% -EPDS scores: no change Null	Low sample size. DHA + AA. No EPA. Longer intervention time. No efficacy.

Nishi et al. (2020) Japan and Taiwan	Acceptable	100 Pregnant women with Edinburgh Postnatal Depression Scale scores ≥9	1800 mg omega-3 fatty acids (containing 1206 mg [EPA]) or placebo	12–24 weeks of gestation for 12 weeks	Supplementation with EPA during pregnancy might alleviate antenatal depression through a mechanism other than anti-inflammation.	Low sample size. only EPA. Low intervention time. Two assessment scale. small efficacy. Need to confirm the efficacy in large sample.
Vaz et al. (2017) Brazil	Acceptable	60 pregnant women were identified as being at risk for PPD	fish oil capsules [1.8 g (1.08 g of (EPA) and 0.72 g of (DHA) acids)] or placebo	week 22–24 of gestation 16 weeks.	Daily supplementation of 1.8 g of n-3 PUFAs for 16 weeks did not prevent maternal depressive symptoms in a sample of Brazilian women.	Low sample size. DHA < EPA. Longer intervention time. No efficacy. No prenatal depression screening.
Susanne Krauss-Etschmann et al. (2007)	Acceptable	170 healthy women	DHA 500 mg + EPA 150 mg vs. DHA 500 mg + EPA 150 mg vs.+400 mg MTHF vs. 400 mg MTHF vs. placebo	22 w gestation until Delivery	Null	Low sample size. 4 arms trial. Longer intervention time. DHA < EPA. No efficacy.
[An] Assessment of Methodological Quality using the CASP checklists for randomised clinical trials. Acceptable in quality for the Studies that score above 50% and studies that score lower or equal to 50% are low in quality.						

1.8.1 Evidence For

Numerous studies propose a potential protective effect of omega-3 FAs, particularly DHA, against maternal mental health. Observational studies have shown that low intake of omega-3 FAs, including DHA (260–263), and low omega-3 FA levels (256,263,267–273). are associated with higher depressive symptoms in pregnant women. Furthermore, clinical trials have shown promising results, indicating that supplementation with omega-3 FAs, EPA, and DHA can reduce depression symptoms in the antenatal and postnatal periods (276–282).

1.8.2 Evidence Against

The literature contains conflicting findings. Some studies have not found a significant link between omega-3 FA intake 259,264–266) or omega-3 FA levels (274,275) and anxiety and depression symptoms in pregnant women. Moreover, certain intervention studies have not proved significant beneficial effects of omega-3 FAs supplementation in reducing prenatal depression (255,283–289,292).

Some studies suggest that omega-3 FAs may help prevent anxiety and depression symptoms during pregnancy, but others have conflicting findings. The inconsistency in FAs supplementation results may be due to variations in study designs, the EPA to DHA ratio, and outcome assessment. Future research should address these limitations and further investigate the role of omega-3 FAs in maternal mental health.

1.9 Omega-3 fatty acid and pregnancy outcomes

1.9.1 Preterm birth (PTB) and low birth weight (LBW)

An epidemiological study in the Faroe Islands showed that a high intake of oily fish (high omega-3 FAs) by 50% than that in Denmark had been linked to increased gestation age

and improved pregnancy outcomes (293). This association has been recently examined in several observational and experimental studies, including clinical trials. Mostly, these studies were unsuccessful in confirming the positive impact of omega-3 FAs on preterm birth (140,294–298). However, it is well-recognised that high omega-3 FAs levels and intake in women during pregnancy might increase gestational age and prevent preterm delivery and LBW (73,141,299–301). It was evident that omega-3 FAs have a role in reducing the neonatal death and stillbirth rate (141), preventing children's allergies (302–304) and enhancing neurocognitive growth (304).

DHA seems to play a vital role in supporting ideal pregnancy outcomes, most remarkably decreasing the rate of early preterm birth and increasing gestational age (139,305). A systematic review indicated that omega-3 FAs supplements in pregnant women had increased gestational age, reduced LBW and preterm delivery (73).

Three RCTs have examined the adjustable doses for omega-3 FAs supplements on lowering LBW and reducing preterm birth risk (139,285,306) and found positive effects when using 800 to 600 mg/day of DHA supplements. However, other trials with the same design did not find any positive role with regard to a 400 mg/day DHA supplement in preventing LBW and preterm birth (307).

DHA supplementation during pregnancy has a positive effect on birth weight. In contrast, DHA and EPA or EPA and alpha-linolenic acid (ALA) supplementation did not impact birth weight risk (302). However, two meta-analyses to investigate the impacts of omega-3 FAs on pregnancy outcomes failed to find any improvement in recurrent preterm birth (302,304). A review assessing the association of omega-3 FAs with pregnancy outcomes

found that DHA plus EPA or pure DHA) supplementation had a minor positive effect on increased gestation age and birth weight but no effect on preventing preterm birth (302).

A Cochrane Review published in 2018 analysed outcomes from nine RCTs involving over 5,000 pregnant women, indicating a significant impact of omega-3 FAs supplementation on preterm birth outcomes in 11% of preterm birth and 42% of early preterm birth (308). After that, two large trials aimed to measure the potential benefits of omega-3 FAs supplements on early preterm birth development were published. One of these studies, in Australia, that included more than 5,000 pregnant women failed to report any improvement in premature birth with 100 mg/d of EPA and 800 mg/d of DHA omega-3 FAs supplementation (309). However, a subsequent analysis of the study data revealed that a subgroup of the study sample, including those with low omega-3 FAs at baseline, might benefit from such an intervention (310). The same result was replicated in a recent trial in the United States involving more than 1000 pregnant women, aiming to test the effects of omega-3 supplements on early preterm birth (311); women were randomly allocated to take 200 mg or 800 mg supplements. They found that pregnant women who started the trial from a low baseline DHA level and took the higher dose benefited from a lower early preterm birth rate.

1.9.2 Gestational diabetes

Evidence shows that women with GDM had lower omega-3 FAs and hypovitamin levels (312–314). RCTs have appeared to suggest that omega-3 FAs supplements could improve pregnancy outcomes and lower the risk of GDM (315). Women with (GDM) can reduce inflammation and oxidative stress by consuming EPA and DHA (316). However, studies on the omega-3 FAs effects on GDM reported conflicting results (315,317,318).

A clinical trial evaluating the omega-3 FAs efficacy on GDM found a positive impact in terms of controlling fasting plasma glucose and decrease of inflammation markers (C-reactive protein) and oxidative stress markers (malondialdehyde), as well as a lower rate of newborn hospitalisations, compared to the placebo (315).

These are probably because the omega-3 FAs influenced the pregnant women's metabolic biomarker and improved the uptake of bile acids (319,320). Omega-3 FAs inhibit inflammation by decreasing adhesion molecules, leukocyte chemotaxis, leukocyte-endothelial interaction, inflammation-induced cytokines, and T-cell reactivity (321). The mechanisms behind their anti-inflammatory actions involve modifying cell membrane fatty acid composition and inhibiting the pro-inflammatory transcription factor NF-κB activation (322).

Omega-3 FAs supplementation (1g/ day) for six weeks in patients with GDM confirmed a significant reduction in inflammation markers and ideal pregnancy outcomes (315). However, others reported that six weeks of omega-3 FAs supplementation in pregnant women does not significantly influence fasting plasma glucose but did reduce HOMA-IR and serum insulin levels (318). Conversely, 800 mg/d of DHA supplement during the middle stage of pregnancy shows no effect on pre-eclampsia or GDM (323).

Vitamin co-supplementation with omega-3 FAs might significantly improve pregnancy outcomes in women with GDM (324,325). Furthermore, in a six-week clinical trial using a mixture of vitamin E and omega-3, the FAs supplement improved metabolic markers in GDM women (326). Similarly, a recent meta-analysis including four randomised control trials to evaluate the beneficial role of co-supplementation on women with GDM showed a reduction in oxidative stress and glycaemic index (327), while another meta-analysis

that aimed to investigate the role of omega-3 FAs supplementation on GDM patients found an essential decrease in C-reactive protein, HOMA-IR, and fasting plasma glucose in patients with GDM (328).

Studies have shown that there may be variations in the FAs level of women with GDM compared to healthy pregnant women. For example, in observational studies, women with GDM had low unsaturated fatty acids (USFAs) and increased saturated fatty acids (SFAs) levels compared to healthy pregnant women (329,330). This shift towards SFAs has been associated with insulin resistance, a key feature of GDM (330). However, studies focused on specific subclasses of SFAs did not show consistent results. For instance, recent studies conducted on Chinese women found that palmitic acid (C16:0) and myristic acid (C14:0) levels were linked with GDM compared to control healthy women (331,332). In other studies, odd-chain heptadecanoic acid (C17:0) and pentadecanoic acid (C15:0), and even full chain SFAs had converse relationship with GDM (333). These findings were also reported in studies of T2D (334) and cardiovascular diseases (335). In addition, these findings were also supported by the results of Chinese studies, which found that higher levels of long-chain SFAs were linked with a high risk of developing GDM (336).

Decreased levels of omega-3 FAs, such as DHA and EPA, increased LA omega-6 FAs, and decreased monounsaturated FAs (MUFAs), such as oleic acid, were also observed in women with GDM. MUFAs have been shown to have a beneficial influence on insulin sensitivity and glucose metabolism (330).

1.9.3 Preeclampsia

Omega-3 FAs levels are shown to be low among pregnant women with PE (337,338). While several RCTs have reported the positive impact of omega-3 FAs in decreasing PE during pregnancy, the outcomes are inconsistent (296,323). An observational study that reviews the association between omega-3 FAs and PE indicates that a 1% increase in omega-3 FAs levels in pregnant women can decrease the risk of PE by 24% (339).

Reviews have yet to emerge to examine the effectiveness of omega-3 FAs supplements for pregnancy induced hypertension (141,294,340). For example, Horvath et al, (294) reported no significant influence on the risk of PE. However, this review was based on a small-sized sample of pregnant women (328 women from 2 trials) (140). Moreover, a meta-analysis (Imhoff-Kunsch et al., failed to indicate a possible role for omega-3 FAs supplements during pregnancy in the development of gestational hypertension (141). Furthermore, a review includes 6 clinical trials reviewing the effects of omega-3 FAs supplementation involving 4,130 pregnant women failed to show improvements in PE (340). In a recent meta-analysis (341), the researchers assessed omega-3 FAs supplements for preventing PE. They reported a weak positive effect on reducing PE and pregnancy-induced hypertension.

1.10 Vitamin D

1.10.1 An overview

Vitamin D, B12, and folate are commonly found in fish and are often supplemented during pregnancy. These nutrients can influence the relationship between fish consumption, maternal depression, and pregnancy outcomes; therefore, understanding their role is

crucial for accurately interpreting the connection between fish intake and maternal and fetal health.

Fat-soluble vitamin D is involved in various metabolic processes in the body. It can help maintain healthy bone and phosphate levels and calcium homeostasis (342). It is classified as a neurosteroid hormone essential for brain homeostasis as it regulates gene expression in a comprehensive series of cellular signalling pathways (343). It can be found in limited food sources, e.g., oily fish, beef, egg, and fish liver oils (344). However, sunlight is the central supplier of vitamin D in the human body (342). Vitamin D3 (25-hydroxy V-D3) is the inactive form of vitamin D synthesised in the liver through hydroxylation.

The vitamin D3 production process begins from the skin through the absorption of ultraviolet B radiation from sunlight through the 1a-hydroxylase enzyme, which stimulates the conversion of a cholesterol metabolite (7-dehydrocholesterol) to vitamin D3 through the action of the 1a-hydroxylase enzyme (345). The kidney hosts the extra hydroxylation process to form the active type of 1,25-dihydroxy vitamin D3. Hence, the vitamin D levels in the body depend on the length of the photoperiod (346). The excess of 1a-hydroxylase enzyme and vitamin D receptors which act to form the vitamin D across brain tissue has been proposed that vitamin D might be vital in the integrity of mental and biological pathways (342).

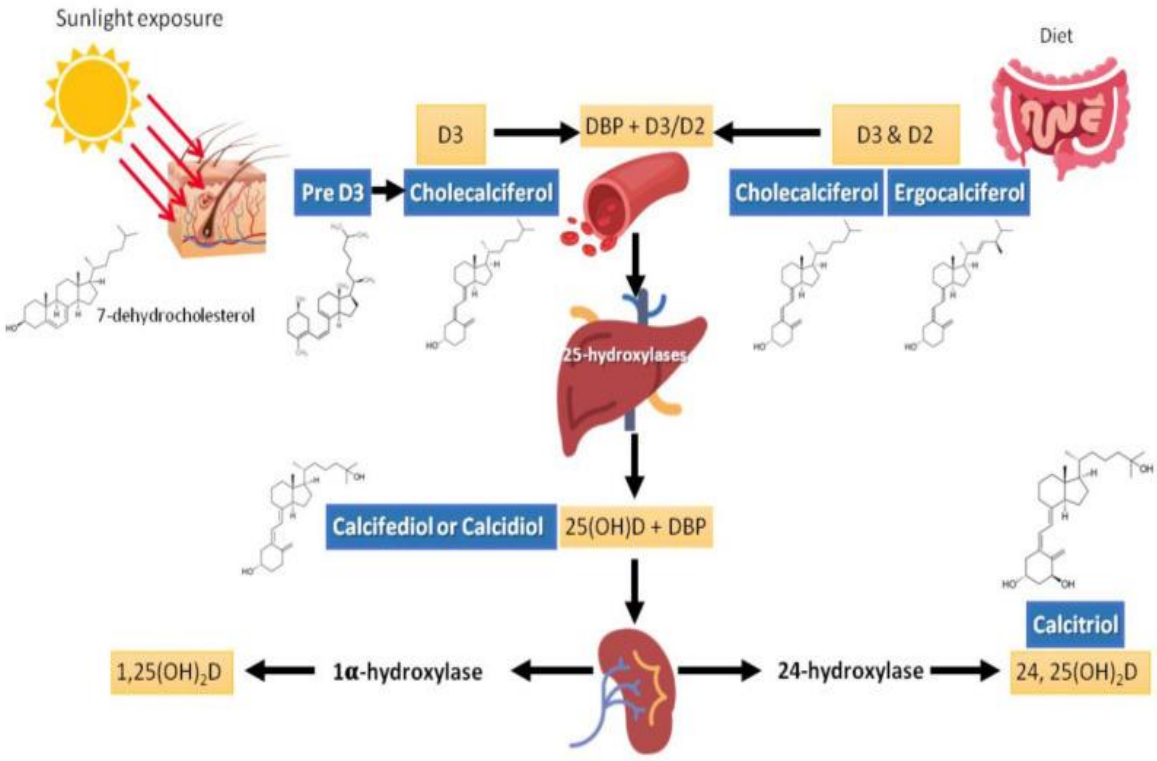

Figure 1-3 The synthesis of vitamin D3 (cholecalciferol, D3).

1.10.2 Vitamin D deficiency and ill health problems

It is well-recognized that low vitamin D levels in early life are linked to rickets (347), osteoporosis (348), osteomalacia, and fractures (349), in adults. Studies show that these bone metabolism disorders could be prevented by supplementing calcium and vitamin D (350). Several findings have confirmed that low vitamin D status is linked with autoimmune and infectious diseases (351) and multiple sclerosis cases (346), T2D (352), cardiovascular disease (353), and breast and colorectal cancers (354). Conversely, the findings of these studies are not consistent.

Vitamin D's role in brain function has been examined in observational studies. Stumpf et al, (355) were the first to describe the vitamin D role on brain function. Furthermore, the human brain can convert 25-hydroxyvitamin-D into active vitamin D due to the existence of the 1α-hydroxylase (1α-OHase) and 1,25-dihydroxy vitamin D3 receptor (VDR) discovered by (356,357).

1.10.3 Dietary recommendations for vitamin D

Although there is no standard international procedure for defining vitamin D deficiency, some organisations provide guidance that states that a level of 30 n/ml is sufficient. Meanwhile, 21 to 29 n/ml is considered insufficient, and a level below 20 ng/ml indicates a deficiency in vitamin D (358).

According to Munns et al, (359), the Endocrine Society recommends a vitamin D status value of 40 ng/ml. The Institute of Medicine (IOM) suggests 20 ng/m (360). European Food Safety Authority (EFSA) report that no specific vitamin D dietary intake is recommended for pregnant women. The IOM recommends 600 international units (IU/d) for the general population and during pregnancy to avoid vitamin D deficiency (360). Furthermore, a review aiming to assess the positive role of vitamin D supplements for pregnant women indicates unclear outcomes regarding whether vitamin D supplements should be prescribed as part of regular maternal care (361). However, a few clinical trials indicate the benefit of 1000–2000 (IU/day) vitamin D supplements on pregnancy outcomes and daily doses of 4000 IU for women with vitamin D deficiency (362).

1.10.4 Pathophysiology of the role of vitamin D, vitamin B12 and folate in depression

The significance of vitamin D deficiency goes beyond just maintaining healthy bones. It is crucial in reducing the risk of several health disorders, such as cardiac disease, T2D, infectious and autoimmune diseases, and various cancers. Hence, it has received significant attention (363). One hypothesis is that vitamin D deficiency might raise depression symptoms in the general population (364). Emerging studies indicate that lower vitamin D levels have been linked with a high rate of antenatal depression (365).

There is a possible connection between depression and anxiety and vitamin D deficiency through various biological processes, such as the hypothalamic-pituitary-adrenal axis and insulin/serotonin mediated pathways (356,357). Although the role of vitamin D status and depression remains unknown, insufficient levels of vitamin D can impair the functioning of vitamin D receptors, leading to a disturbance in those hormonal and biological processes that play a crucial role in regulating mood disorders within the human brain (366).

Low vitamin D levels are linked to a high rate of inflammatory cytokines, while an increased level of vitamin D acts as a defence element in the association between depression and pro-inflammatory cytokines (367). Several findings have shown an association between high inflammation status and depression (368–370). During pregnancy, vitamin D controls the function and growth of the placenta and the fetus (371). In contrast, it has been noted that women with vitamin D deficiency during pregnancy may experience unfavourable pregnancy outcomes (372,373). The typical appearance of the vitamin D receptor with its synthesis enzymes in the central nervous system suggests that

the low levels of vitamin D could be related to a progression of several psychiatric and neurodegenerative disorders such as depression (374,375). Low vitamin D levels have been classified as a trigger for depression in adults and in prenatal depression due to multilayered functions (376,377).

1.10.5 Vitamin D levels and depressive symptoms in the general population:

1.10.5.1 Epidemiology and observational studies

A meta-analysis of about 32,000 males indicates that vitamin D deficiency is conversely linked with clinical diagnoses of depression disorder (378). These studies prove a correlation between high depression symptoms and low vitamin D (379). However, the majority of these studies have come mainly from research conducted in the ageing group, healthy males, and in populations with certain chronic diseases, but only a few studies have involved females (379–387), Conversely, these outcomes have not been replicated in other findings (388–391). Likewise, an extensive epidemiological study of over 1,000 elderly individuals showed that insufficient vitamin D is significantly associated with minor or major depression (383). Similarly, in an observational study that included 56,366 middle-aged U.S. women, a higher dietary vitamin D intake was linked with low depression symptoms (392).

1.10.5.2 Clinical and intervention studies

Several clinical trials that assessed the association between vitamin D supplements and reducing mental illnesses, including depression in different populations, have presented varied outcomes. For example, in two separate trials of healthy adults, vitamin D supplements improved mood through the winter season (393) and enhanced well-being in non-depressed patients (394). Other RCTs involving depressed patients at baseline

indicated a positive role for vitamin D supplementation; these trials demonstrated a reduction in depressive symptoms among obese patients with severe depression rates (395), participants with seasonal emotional disorders (396). A few clinical trials have reported reduced depressive symptoms, while others do not find any beneficial effects.

Two interventional studies examined the influence of vitamin D supplements on depressive rates in females (395,397). The first study found that vitamin D supplementation considerably improved depressive symptoms in the winter months when serum vitamin D levels were equal to 100 nmol/L at the starting point (397), while the other study indicated that there is no reduction in depression symptoms or improvement in emotional well-being in a group of older women compared to a control group (395).

1.10.6 Vitamin D levels and depression and anxiety symptoms during pregnancy

1.10.6.1 Antenatal depression

Numerous observational studies have examined the relationship linking vitamin D status, variations of anxiety symptoms, and prenatal and postpartum depression among pregnant women (398). However, the results have been inconsistent. For example, in the USA, a study including 498 pregnant women evaluating the association between high anxiety and antenatal depression and vitamin D levels in the first trimester of pregnancy showed no associations. However, the association was significant among pregnant women who reported no or low physical activity (399). At the same time, another cohort study among African-American pregnant women (n=178) Cassidy et al, (400) indicated a significant link between insufficient vitamin D levels and depressive symptoms in mid-pregnancy. In the same cohort of women (n=90), Accortt et al, (401) reported an link between vitamin D deficiency and high postnatal depression. Furthermore, data from a

randomised trial undertaken in the USA, Williams et al, (402) indicated a converse association linking vitamin D deficiency in the second trimester with high depressive symptom rates in early and late pregnancy. Furthermore, in the Netherlands, research involving a large sample of pregnant women (n = 4,236) reported a strong relationship linking low vitamin D status and antenatal depression measured in the second trimester (403). Moreover, in a study of Japanese pregnant women (n = 1,745), a lower incidence of depressive symptoms during pregnancy correlated with higher vitamin D intake (364). Two prospective study designs (402,403) and one RCT indicated a significant link between low maternal vitamin D levels and antenatal depression (404). In contrast, only one study showed no such association (399). Conversely, one cross-sectional study reported a higher prevalence of antenatal depression with an average vitamin D level during the first three months of pregnancy (399). At the same time, others found that a high level of vitamin D at an early stage of pregnancy was linked with a reduction in antenatal depression rates (364). Separate studies in each pregnancy trimester have reported similar results of prenatal depression (400,403,404).

1.10.6.2 Postnatal depressions

A longitudinal study in the USA involving 97 postpartum women up to 7 months postnatal reported a strong correlation between low vitamin D concentrations and postnatal depression (405). This was replicated in a study that involved 796 Australian women (406). They found that vitamin D levels measured at 18 weeks of pregnancy were associated with postpartum depression assessed three days after delivery. Likewise, in a longitudinal study in Turkey, the authors suggested a significant link between low vitamin D levels among pregnant women at mid-pregnancy with increased rates of

postpartum depression at seven days of delivery, one month, and six months postnatal (407).

Unexpectedly the authors reported that average vitamin D levels among pregnant Danish women were linked with antidepressant use during the postnatal period. In contrast, Nielsen et al. (2013), in a case-control “Danish National Birth Cohort”, failed to show any correlation between low vitamin D concentrations and perinatal depression in late pregnancy (408). Similarly, an extensive study of Australian women (n = 1,040) failed to report any association between vitamin D status in cord blood with postnatal depression at delivery or after six months postnatal (409). However, in other longitudinal studies involving Chinese women (n = 213), reported an association between postpartum depression assessed three months after childbirth and vitamin D levels considered 24–48 hours after delivery (410). A randomised clinical trial involving 169 Iranian pregnant women indicated that 2,000 IU of vitamin D3 per day through late pregnancy effectively reduced antenatal depression and depression at 4 and 8 weeks postpartum (404). A significant link between high postnatal depressive symptoms and low maternal vitamin D levels was reported in three studies (406,407,410) and one intervention study (404). Conversely, others indicated no such association (401,408,409).

Inadequate vitamin D levels (<48 nmol/L) are linked with a high rate of depression symptoms three days after childbirth (406). In contrast, a sufficient amount of vitamin D was associated with low depression symptoms at three months (410), one week, six weeks, and six months after childbirth (407), four to eight weeks after childbirth (404), and 1 to 7 months (405) after childbirth. Moreover, vitamin D deficiency was also linked with

a high rate of depressive symptoms, but not among women with diagnosed major depressive disorders (402).

1.10.7 Vitamin D status and pregnancy outcomes

1.10.7.1 Small for gestational age (SGA), preterm birth (PTB) and low birth weight (LBW)

Low vitamin D levels during pregnancy can lead to various health conditions for the mother and her child, including metabolic disorders in the developing fetus. Although this area of research has been extensively reviewed, further research is needed (342). Early observation of the association linking vitamin D and possible poor neonatal outcomes, including preterm birth, SGA, and LBW, indicates that it is connected with a high risk of several disorders in later life (411). For example, vitamin D deficiency and LBW were reported in extensive cohort studies involving 7,098 pregnant women (342). The results were replicated in observational research (412,413). However, other studies have rejected this relationship (414–416). For example, two observational studies showed that women with normal vitamin D status are at low risk of having LBW (417,418). Contrarily, others have confirmed that vitamin D deficiency in women is linked with macrosomia (419,420).

In addition, a meta-analysis found no positive effects of vitamin D supplements on LBW (421) or the risk of preterm birth (422,423). This conclusion was supported by an observational study investigating vitamin D levels during early pregnancy (424). However, others have confirmed the relationship of vitamin D deficiency with earlier delivery (411,412,425,426) and preterm birth (427–429).

Although observational and clinical trials show a positive link between optimal vitamin D concentrations and gestation outcomes (430,431), further research is warranted.

1.10.7.2 Gestational diabetes

Vitamin D's role in GDM remains questionable, but it might improve glucose tolerance in pregnant women because it plays an essential role in glucose homeostasis through various intracellular processes (432). Primarily, it regulates insulin secretion by pancreatic beta cells and enhances insulin responsiveness in tissues and the liver (433).

Numerous epidemiology studies (434–436) and a meta-analysis which includes 20 observational analyses (437), show that vitamin D deficiency among pregnant women is linked with a high rate of GDM (438). Moreover, in a study, Lacroix et al. indicated that high vitamin D status were linked with a low rate of GDM among pregnant women (439). The same outcome was found in an observational study among Arabic women (440). Conversely, others show no relationship with the prevalence of GDM among different groups of pregnant women from different ethnicities and geographical locations (428,441–443).

In a clinical trial including a group of Iranian pregnant women examining the role of co-supplements in the form of vitamin D and calcium on GDM, Asemi et al, (444) found that the co-supplement had a beneficial effect on the GDM profiles. Similar results were found in a clinical trial using pure vitamin D supplementation in pregnant women (445). However, several clinical trials failed to report any beneficial effects of vitamin D supplements in terms of GDM blood profiles (446–448). Remarkably, in RCTs examining the vitamin D level of ethnically-diverse women in the early stages of pregnancy, a high serum 25OHD is linked with significant high risk of GDM (416,424). The researchers

justified their outcome in terms of confounding factors such as obesity and age. Nevertheless, two meta-analyses found no positive role with regard to vitamin D supplements in preventing GDM (361,449). The variations in the results of these clinical trials could be related to diverse dosages and the intervention time with regard to vitamin D supplementation.

1.10.7.3 Preeclampsia

An extreme inflammatory reaction is one of the multifaceted pathophysiologies of PE (450). The specific mechanism linking low vitamin D levels and PE is not clear; some studies propose that it is involved in the immune response to the fetal–placental unit (451). Vitamin D controls numerous cardiovascular reactions and immunomodulatory pathways. Therefore, PE is assumed to be linked to a low level of vitamin D (452,453).

Several observational analyses (454–457) and meta-analyses (458,459), indicts a relationship between lower levels of vitamin D among pregnant women and a high risk of developing PE (460). Similarly, this result was replicated in a small sample group of pregnant women (n = 280) (461). Conversely, other studies failed to show evidence supporting this conclusion linking vitamin D deficiency to PE (421,462,463). For example, (428) failed to report similar results in a large sample study involving about 5,100 pregnant women.

Nevertheless, RCTs observe the effect of vitamin D supplements as a potential approach to reduce the occurrence of PE. Few clinical trials have reported a positive impact (361,425,464), while others have failed to find a beneficial role in reducing the incidence of PE (421,430,446,465–467). The negative results found in the intervention studies could

be due to the late intervention time during pregnancy, as the supposed protective effects of vitamin D begin in early pregnancy (430).

1.11 Folate and Vitamin B12

1.11.1 An overview

Vitamin B-12 - also called cobalamin and folate – is a water-soluble vitamin intricate in the one-carbon metabolism that certainly exists in diets as glutamate chains (468). The artificial formula for the vitamin B complex is usually used in fortified foods and as nutritional supplements (468).

Folate and vitamin B12 are essential in embryogenesis (469). These B complexes are crucial in fetal and placental development, differentiation, cellular growth, and biosynthetic processes (247). They work as cofactors in altering homocysteine to methionine, essential for creating neurotransmitters and phospholipids (470). Folate, or vitamin B9, is vital for creating purine, RNA, DNA, thymidine nucleotide protein, and lipids in the cellular cytoplasm (470). Folate and vitamin B-12 deficiency can therefore damage cell division and methylation activity, which could consequently interfere with pregnancy and focus outcomes (471).

Low folate and B12 vitamin levels can lead to anaemia, dementia, cognitive dysfunction (472), and nutrition malabsorption (468). Severe folate and vitamin B12 deficiency can cause hyperhomocysteinemia and atherosclerotic vascular disease (473). Diarrhoea or constipation, depressed mood and change in mental status, shortness of breath, loss of appetite, fatigue, and pale skin are classic signs of vitamin B12 and folate deficiency (474,475).

It is progressively more being considered that pregnant women should increase their vitamin B12 and folate levels. A regular dose of 2.6mcg Vitamin B12 is recommended for pregnant women, while 2.4 mcg is suggested for non-pregnant women (476). Several organisations have provided folate and vitamin B12 supplements for pregnant women to ensure an adequate amount is shifted to the fetus (477). The average standard dose of vitamin B12 is commonly known to range between 170 and 250 pg/ml (478). At the same time, vitamin B12 deficiency is described as existing at a level lower than 150 (pg/ml) (479).

1.11.2 The Role of Folate and Vitamin B12 in Depression in the General population

Growing evidence indicates that depression might be linked to folate and vitamin B12 deficiency. The neuronal hypothesis is the suggested mechanism for these relationships; it means there is a reduced production of dopamine, neurotransmitters noradrenaline, serotonin, and chronic inflammation due to the low status of B vitamins (473,480). The possible biological mechanisms of this relationship have been proposed in terms of concentrating on their role in methionine creation (473). The folate-methylation cycle needs vitamins B9 and B12 as these are crucial in the design of, for example, DNA, red blood cell formation, membrane phospholipids, and neurotransmitters. Low vitamin B12 and folate status damages the methylation of these particles and induces hyperhomocysteinemia (481). Both a reduction in the main neurological molecules and the possible neurotoxic effects of high homocysteine may raise the risk of psychological illnesses (468). Folate and vitamin B12 deficiency cause other neurological appearances such as paraesthesia, neural tube defects, myelopathy, and neuropathy (482).

Folate and vitamin B12 deficiency also cause other neurological appearances such as para<esthesia, neural tube defects, myelopathy and neuropathy (482). Observational studies have shown a clear link between a lower nutritional intake of folate and B12 and the escalation of depression symptoms (483,484). Moreover, low vitamin B12 levels in older people have been linked with higher depression symptoms (474,475), while low folate levels in the general population have been correlated with depression (484,485).

Biologically possible processes of an association between folate and vitamin B12 deficiency and mood disorders, including depression and anxiety, have been proposed, focusing on their role in methionine creation (473,480). The folate-methylation cycle needs vitamins B9 and B12 as essential in the production of, for example, DNA, red blood cells, membrane phospholipids and neurotransmitters (473). Low vitamin B12 and folate levels damage methylation and induces hyperhomocysteinemia (481). Both reduce the neurologically main molecules and possible neurotoxic effects of high levels of homocysteine, which may raise the risk of depression (468).

1.11.3 Folate and vitamin B12 Status and depression and anxiety in the general population

Six decades ago, some researchers suggested a relationship between low levels of folate and B12 in plasma and a high depression rate (486,487); these outcomes propose that supplementation with regard to these vitamins could be used as a treatment to improve depressive symptoms (472).

The relationship between low intakes of folate and B12 and increasing rates of depression symptoms has been indicated in a few studies (483,484). Low folate levels have been linked with depression in the adult age group (484,485). Others indicate that vitamin B12

deficiency in older people is associated with depression symptoms (474,475). A clinical trial (468) showed the beneficial role of vitamin B12 supplementation on adult patients engaged in antidepressant therapy compared to a placebo group.

Furthermore, in a meta-analysis including clinical trials, long-term vitamin B12 and folic acid supplement intake were shown to be beneficial in improving depressive symptoms (488,489). Moreover, Bender et al, (473) reported that depressed patients had a low dietary intake of folate and had considerably lower plasma levels than healthy adults. Another meta-analysis also proposed a link between low folate levels and a high rate of depression symptoms (482). However, the outcome of studies on the link between vitamin B12 and folate remains to be determined. Some studies indicate a link between vitamin B12 in plasma and depression (490–492), but this outcome has not been replicated in other studies (485).

1.11.4 Folate and vitamin B12 levels and pre-and postpartum depression

As has been well described earlier, the expected mothers are at significant risk of nutrient deficit during pregnancy and after childbearing due to the high requirements of the growing fetus and the mother's biological needs (247,493). Numerous studies have recently stated an association between micronutrient deficiency and antenatal depression (277,494) and postnatal depression (401,495). Vitamin B12 and folate deficiency, which affect brain functions related to mood regulation, are possible factors in terms of depression developing among pregnant women (247). Lower vitamin B12 and folate levels in the blood during pregnancy were associated with a risen risk of depression (496,497), while some observational studies on the impact of vitamin B12 and dietary folate intake (481) and levels in erythrocytes, showed no significant relationship with

antenatal depression (498) and postpartum depression (478,481,496,498). Randomised clinical trials (478) show an inverse relationship between folate supplement intake in the second and third trimesters with antenatal and postnatal depression.

1.11.5 Folate and vitamin B12 levels and pregnancy outcome

Folate and vitamin B12 deficiency during early pregnancy are linked with poor pregnancy outcomes and congenital disabilities such as miscarriage (499), LBW (471), and neural tube defects (NTDs). It could also lead to PE (500), GDM (470) and preterm birth (501,502).

The role of vitamin B12 and folate status on GDM during pregnancy has appeared as an area of attention (476,503). Several studies have shown that homocysteine status is considerably raised in pregnant women with GDM (504,505). However, other findings have shown that folate and vitamin B12 status during pregnancy were comparable among women with or without GDM (505,506). Moreover, the rate of GDM increased with low vitamin B12 among women (476). In a cohort study, a low intake of folic acid in women in the early stage of pregnancy was linked to a high GDM rate (477). Moreover, evidence indicates that high folate levels during pregnancy and a vitamin B12 deficiency were linked to an increased GDM rate (479).

Several experimental findings have shown a correlation between low levels of vitamin B12 among pregnant women and LBW or SGA in vegetarians (471,507) and the non-vegetarian population (508). However, the relationship linking vitamin B12 and LBW needs to be fully understood (509).

1.12 Healthcare system in Oman (Antenatal Care Service)

Oman is the second largest country in the Arabian Gulf on the Arabian Peninsula. In 2019, the national census in Oman estimated the Omani population to be 4.62 million. Of these, 2.66 million were Omani (57.5%), while 1.96 million were expatriates. About 64% of Omanis were aged below 30 years, and 15% were children under five. Only 3.6% were over 65 (median age: 22). In 2019, approximately 115,658 individuals resided in the AL Buraimi Governorate (510). Thus, Oman has pyramidical-like inhabitants structured with the preponderance of youth.

The Ministry of Health (MOH) offer universal free healthcare services for all Omanis. It provides healthcare to the country's citizens at various levels through its multiple facilities, such as primary and comprehensive health centres. It also operates regional referral hospitals that provide secondary-level services (511). Despite Oman's impressive achievements in offering healthcare services over the years, it still needs to improve its healthcare system's sustainability. These include meeting increasing consumer expectations, the need for more resources, and the constraints in its capital infrastructure and workforce (512).

Concurrently with universal free services, Oman has a flourishing private healthcare system that caters for the expatriate population. Primary healthcare centres are considered the initial port of call to all healthcare divisions in Oman. In the Al Buraimi Governorate, as soon as a woman becomes pregnant, standard maternal healthcare services are provided in the antenatal clinics of 6 local primary care health centres and one polyclinic, each offering healthcare in response to their particular condition (513). In the antenatal clinics, every pregnant woman in Oman is supplied with an antenatal ‘green

card' which contains the patient's information, outcomes of previous pregnancies, personal and family medical history, and the medical notes of follow-up antenatal visits. Each pregnant woman was encouraged to take a multivitamin dietary supplement (514). In Oman, all pregnant women are advised to take dietary supplements, including 500 mg/d of folic acid and 600 IU/day of vitamin D3 (514). During childbirth, it records pregnancy outcomes such as delivery information, the Apgar score, and the baby's birth weight. In brief, six antenatal visits to health centres are required during normal low-risk pregnancies. In contrast, higher-risk pregnancies are referred through specific protocols to maternal clinic services in secondary or tertiary hospitals, depending on the severity of the case. After childbirth in the hospital, women are followed up by staff in their local health centre for postpartum care between 6 and 8 weeks. However, no screening assessment in terms of antenatal care protocols exists to identify women with antenatal depression within the MOH institutions in Oman (61).

1.13 Health problems in pregnancy in Oman

Oman is in a health transition stage with a high level of morbidities, one of the characteristics of newly developing nations. The MOH in Oman has made impressive improvements in terms of health status indicators over recent years. For example, the under-five year's mortality rate (U5M has been reduced from 47 in 1990 to 9 in 2011 (515). This success has been broadly acknowledged and admired by several global institutions. The WHO classified Oman as one of the best ten associate states in the 2000 World Health Report (516). Despite these markers of significant progress in the health services sector, some alarming figures have appeared as new challenges for promoting mother and child health in Oman. For example, LBW is a significant indicator of newborns'

health status and childhood mortality. The LBW rate has been increasing in Oman in the last few decades. A cross-sectional hospital-based study including 534 singleton live births in Muscat (517) indicated that the incidence of LBW and preterm birth was 13.7% and 9.7%, respectively.

In terms of GDM, in an observational hospital-based study including 5,394 Omani pregnant women, reported that about 24.4% had GDM (518). At the same time, the annual health statistics report of the MOH in Oman indicates that the GDM rate among pregnant Omani women has increased sharply from 4.8% in 2012 to 15.1% in 2017 (519).

Maternal obesity is also considered a concerning health issue among Omani pregnant women. Two observational studies aimed to assess prenatal obesity among 3,352 Omani women (520,521), which indicated that 34% were obese and an additional 31% of the study sample were overweight. The authors found that maternal obesity in Omani women carried a high risk of PE (4%), GDM (15.7%), fetal macrosomia (6%), CS (20.8%) and a high rate of miscarriage (11.9%).

In addition, several studies have indicated a higher risk of fetal macrosomia as a pregnancy outcome in the case of high-parity, obese, older, and GDM Omani pregnant women (522). In contrast, Omani women with low nutritional status (BMI < 18 kg/m2) were linked with a high rate of LBW (523). In a recent national cross-sectional survey in Oman to estimate anaemia and micronutrient deficiencies among Omani pregnant women, Petry et al, (524) showed that the prevalence of anaemia was 27.8%. In contrast, iron deficiency was 24.8%, vitamin D deficiencies was 16.2%, folate was 11.6%, and vitamin B12 was 8.9%.

Over the past twenty years, research has revealed significant maternal vitamin D deficiency in Oman. The micronutrient status survey conducted in 2004 shows that 21.4% of the women of childbearing age included in the study were deficient in vitamin D (<27.0 nmol/L) (525). A study including 103 pregnant Omani women, showed that 33% of the women had a vitamin D deficiency (526). Furthermore, in a study involving 41 pregnant Omani women, reported that all the women had a vitamin D deficiency (527). In 2017, the last National Nutrition Survey in Oman showed that 43% of women of childbirth age had vitamin D deficiency, while deficiencies in vitamin B12 and folate were reported at 11.6% and 8.9%, respectively (524).

1.14 The rationale of the study

The discussion mentioned above via observational and clinical trials has assessed the possible effects and potential benefits of omega-3 FAs intake in depression aetiology during pregnancy (117). However, the exact relationship between antenatal depression and omega-3 FAs consumption remains uncertain. If omega-3 FAs intake is quantified and found to be valuable in mitigating depression, one implication would be that depression could be conceptualised as a medical or nutritional disorder. In Omani society, this would have implications for the prevailing belief that depression is triggered by supernatural forces (528). The general stigmatisation of people with psychiatric disorders has been documented (529).

To date, pharmacological treatment is the main intervention available to people with depression in Oman. Clinical evidence-based studies indicate that treatment declines are expected in patients with depression, consequently leading to a high dropout and low compliance among people receiving antidepressant treatment (530,531). Combination

treatments for depression are well-tolerated and lead to increased remission rates (532,533). However, despite the accessibility of many antidepressants, there is a need to seek a natural, safe and effective agent for depression (534). If nutrition is a potential cause, dietary supplements may complement existing treatments. In the UK, the National Institute for Health and Care Excellence (NICE) guidelines state that it is essential to seek alternative treatments for depression before resorting to pharmaceuticals due to the cost-benefit ratio being too high (3) and unwarranted side effects (62). This includes cognitive-behavioural therapy (CBT) and interpersonal therapy (IPT), effectively addressing underlying psychological factors without potential side effects (3).

Due to the rise in the economic and social consequences of depression and the fact that existing pharmacotherapy is not sufficient for depressive symptoms, there is a need to mitigate depressive symptoms using compounds and supplements with fewer side effects. Therefore, omega-3 FAs have been hypothesised to be an additional treatment for depressive disorders.

In the case of Oman, a country undergoing rapid changes and development, this study will provide empirical evidence that one of the most commonly encountered emotional disorders, depression and anxiety, during pregnancy can be effectively alleviated by increased dietary seafood intake during pregnancy. It is hoped that the findings will complement data emerging from other regions of the world. In Oman and other Gulf countries that share a similar language (Arabic) and culture, there are currently no available data or studies that discuss the link between seafood consumption or omega-3 FAs intake and maternal depression and anxiety symptoms and pregnancy and neonatal outcomes.

Numerous studies have proposed that ethnic backgrounds and cultural experiences might influence patient-based outcomes (535). In patients from Arabic-speaking nations, it is necessary to highlight the different beliefs, norms, values, and cultures that could affect the intervention treatment of depression. Most studies on the positive effect of omega-3 FAs intake on emotional health disorders (including depression) and pregnancy and neonatal outcomes have been conducted in Western countries. However, variances in values and cultural beliefs mean that the subjective experiences of pregnant Omani women (and women in many other Arab countries that share the same values, culture, and ideas) should be the subject of separate studies that determine whether or not antenatal depressive symptoms are linked with insufficient seafood or omega-3 FAs intake within such a cultural context.

The study's objectives are thus to establish information about the incidence of anxiety and depression symptoms and pregnancy outcomes among pregnant Arabic-speaking women to evaluate the potentially valuable role of omega-3 FAs from seafood intake on prenatal depressive and anxiety symptoms and pregnancy outcomes. Another net expected product of the study of the proposed cohort is to raise awareness about a healthy balanced diet and lifestyle for pregnant women in Oman. Confirmation of the positive results of the study could help healthcare policymakers in Oman establish guidelines and recommendations for increased intake of omega-3 derived from oily fish in the case of pregnant women and food fortification with omega-3 in different products, targeting other age groups.

Reducing the impact of emotional disorders and pregnancy and neonatal outcomes in women is very important. Enhancing the quality of life for pregnant women suffering from

antenatal depression and the people around them will aid in improving the socioeconomic status of the community. The long-term outcomes of this research project will include reducing the healthcare costs associated with depression and pregnancy and neonatal outcomes and raising awareness with regard to healthy lifestyles for treating depression in the Omani community.

The study will use routine clinical investigations as part of the data collection process. The data collection will consist of regular assessments. This study will, therefore, constitute ‘natural observation’ since patients are routinely examined with such assessment measures. The empirical data derived from this study will aim to strengthen ongoing clinical services and provide much-needed evidence-based intervention for this particular population. Thus, besides these benefits, there will be no demands on local health services. The study will be a naturalistic observation of routine clinical services. The data collection will, therefore, increase the quality of the data gathered without infringing on other clinical services or therapist-patient relationships.

Previous studies investigating the association between fish and omega-3 FAs intake and levels with maternal mental health are subject to several methodological limitations that may influence the findings. These limitations include the uses of observational designs, variations in measuring omega-3 levels and dietary assessment methods to estimate omega-3 FAs intake, disparities in the assessment of maternal mental health (specifically depression and/or anxiety), small sample sizes, the potential for confounding variables, and challenges related to participant adherence to study protocols.

This study aims to address most of the weaknesses of the previous studies and fill a gap in our knowledge of anxiety symptoms during pregnancy and antenatal depression, and

pregnancy and neonatal outcomes with nutrition research in the case of Arabic pregnant women. This study is anticipated to generate precise data to help expand the management of patients afflicted with anxiety disorders. This will be the first study to address the relationship between nutraceutical omega-3 FAs, psychological health issues, and pregnancy and neonatal outcomes in the Gulf region and the Middle East. It will also add to the current knowledge base in nutrition and mental health diseases.

The proposed study was the first in the Sultanate of Oman to bring together a multidisciplinary group of scientists in the areas of lipidomic and mental health disorders from highly reputable institutions to assess the relationship between seafood and omega-3 FAs intake as a potential cure for antenatal anxiety and depression symptoms. The research project is being carried out through collaboration among various international and local institutions. These include the Department of Digestion, Metabolism and Reproduction, Faculty of Medicine, Imperial College London; the Ministry of Health, Oman; the Department of Nutrition, Al-Buraimi Hospital, Oman's College of Medicine, and the Faculty of Medicine at Sultan Qaboos University.

1.15 Research aims

The overall aims of the study are to examine the potential association between fish consumption, omega-3 FAs intake and maternal and (cord) erythrocytes' FAs levels with maternal mental health and pregnancy outcomes among Omani pregnant women.

1.15.1 Objectives

In a cohort of pregnant Omani women:

To better understand the relationship between anxiety and depressive symptoms and pregnancy outcomes, a series of assessments can be conducted. These include:

1_ Determine the incidence of prenatal depression and anxiety.

2_ Quantify fish and Omega-3 FAs intake and measure FAs levels in erythrocytes.

3_ Measure serum levels of vitamin D, B12, and folate.

4_ Assess the inter-relationships between these factors and pregnancy outcomes.

By conducting these assessments, we can gain valuable insights into the impact of these factors on the health of both the mother and the developing fetus.

1.15.2 The main scientific hypothesis

Low maternal FA intake and low content of EPA and DHA omega-3 FAs in erythrocytes are associated with a high rate of prenatal depression and anxiety symptoms among Omani pregnant women.

Chapter 2: Methodology

2.1 Study Design and Participants

This longitudinal cohort study recruited 300 pregnant Omani women receiving maternal care at Al Buraimi Hospital, Oman. The level of depression and anxiety was assessed at 8-12 and 24-28 weeks of pregnancy using a modified self-administered Arabic version of the Edinburgh Postnatal Depression Scale (EPDS) translated by Mohammed et al (48). A validated food-frequency questionnaire (FFQ) has been used to measure the dietary consumption of seafood and DHA and EPA omega-3 FAs in pregnant women (536). Vitamins levels (Vitamin D, Vitamin B12, folate) were assessed in the medical lab of Al Buraimi Hospital. Erythrocytes and plasma were isolated and stored in a -80°C freezer for further analysis. In brief, the patient's electronic medical records were reviewed to obtain antenatal, intrapartum, and postpartum data. Maternal characteristics and complications were evaluated using general health questionnaires. Pregnancy and neonatal outcome records for mothers and their offspring were collected and used to realise the aims and objectives of the study. The Board of Ethical and Research Committee in the Ministry of Health in Oman approved the study protocol on the 1st of July 2019 (MoH/CSR/19/9668) (The ethical approval is attached in Appendix 4).

2.2 Sample power calculation

The sample size of this cohort should be sufficient to achieve a descriptive sample of pregnant Omani women to measure the incidence of depressive and anxiety symptoms and the role of omega-3 FAs intake on prenatal depression and anxiety symptoms and pregnancy outcomes. The determination of the appropriate sample size is based on a population of 1,800 pregnancies in Al Buraimi Hospital (519). The sample size was

determined using the Pearson r method to calculate the correlation coefficients using the G* Power software 3.1.1 calculator (537). A sample size of 138 was required to achieve statistical adequacy based on a medium effect size of f=0.3, an α-level of 0.05, and a power of 0.95 (538). Given the expectation of a 40% non-response rate (N = 56), 40% presented missing data for confounder variables or pregnancy outcome, for example, delivery at a different hospital (N = 56). Thus, it was essential to raise the sample size to 300 pregnant women to confirm adequate numbers to ensure the study's reliability.

The study's power calculation considers its ability to detect significant changes in anxiety and depressive symptoms that can be attributed to the fish and omega-3 FAs intake. To ensure adequate statistical power, a sample size of 300 pregnant Omani women has been selected, counting expected non-response rates and missing data. Therefore, the study can reliably assess the relationship between omega-3 FAs intake and levels with prenatal mental health outcomes, and measure changes in anxiety and depressive symptoms among the target population.

2.3 Inclusion and exclusion criteria

The participants were recruited from the Al- Buraimi Hospital antenatal clinics and the Primary Health Centre in Al Buraimi, which serves the local community of the Al-Buraimi Governorate. The inclusion criteria for the female participants in the study were as follows: (i) aged between 18 and 45 years of age; (ii) 8-12 weeks gestation of single pregnancies; (iii) expecting to continue prenatal health care in the antenatal clinic at Al Buraimi Hospital; (iv) agreeing to participate in the study and are residents of Al Buraimi, Oman.

Pregnant women who were non-Omani, or with severe health conditions (e.g., cancer, heart disease, and any infectious disease such as Hepatitis C and B carriage, human

immunodeficiency virus (HIV), cirrhosis or other chronic liver diseases, comorbid substance abuse, or psychotic, bipolar or obsessive-compulsive disorder, or any other neurological or psychiatric conditions that may affect depressive disturbances, were excluded. Also excluded were women with a record of alcohol or drug dependence in the earlier six months; those undergoing anticoagulant therapy or taking mood-altering medications; those aged <18 years; high-risk pregnancies and fetal anomalies; and pregnant women who have experienced multiple births.

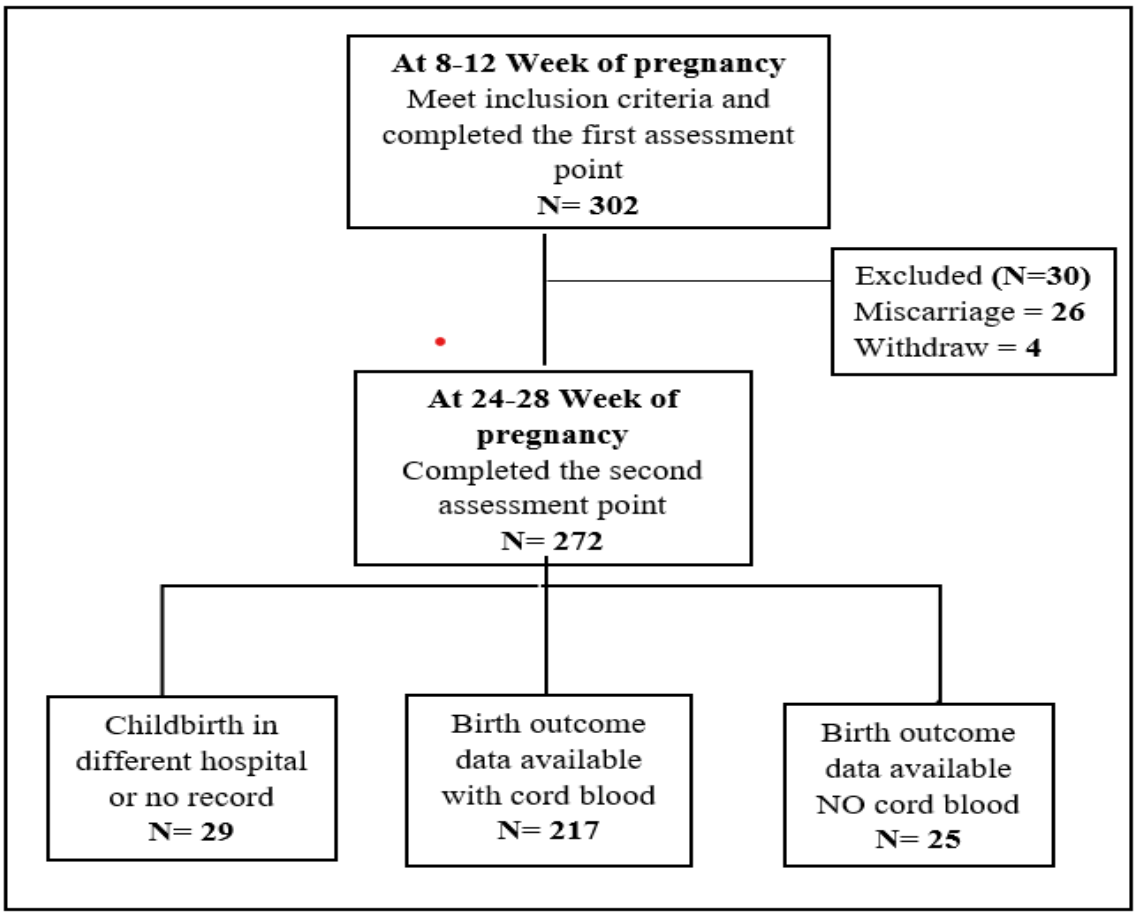

Figure 2-1 Flow chart visualising the study design and sampling points.

Table 2-1 Study assessment points:

	At 8-12 Week of pregnancy	At 24-28 Weeks of Pregnancy	Childbirth
Blood Analysis	Maternal Fatty Acids analysis (RBCs) Vitamin D, Vitamin B12 Folate		Cord blood Fatty Acids analysis (RBCs)
Depression and Anxiety symptoms	The EPDS scale	The EPDS scale	
Omega-3 fatty acids dietary intake	Food Frequency Qs 7-day Food Record		
Sociodemographic and Maternal Characteristic	General Health Qs Patient Medical notes		Maternal and neonatal outcomes

2.4 Data collection

The participants were recruited from the Al Buraimi Hospital antenatal clinics and the Primary Health Centre in Al Buraimi. The PhD student (PI) oversees data management and data quality. The quality control of data in this study was performed by describing and applying criteria used throughout the study; for example, applying descriptions used for the data tables or data entry procedures, determining codes and abbreviations, specifying unit measurements and relevant identifying metadata before collection. Furthermore, to eliminate errors through data entry processes, dual data entry techniques were used to check any entry differences and to identify other mistakes. Moreover, appropriately designed spreadsheets or databases were used for data collecting in the study, using consistent terminology within the database and atomised data.

Measures were taken once the data had been entered to assure the quality: for example, checking for any missing, impossible, or anomalous values, sorting data fields and checking for discrepancies to look for outliers, and implementing simple statistical

measures such as standard errors and means. This was also applied during the data analysis to confirm that everything was correct during the transformation.

The confidentiality and privacy of the subjects were ensured by double coding, and data access restriction was used to prevent traceability. Dual coding, rather than who consented to participate in the study, patient confidentiality, as the latter does not allow clinical monitoring and subject follow-up. The sample of pregnant Omani women who agreed to participate in the survey were given a computer-generated code number which does not carry any personal identifiers (first coding). The subjects were then re-coded with another computer-generated number (2nd coding). All the coded data generated from the study were kept in a dedicated computer and protected with a password that was changed regularly. The data were entered and analysed using statistical analysis SPSS software.

2.5 Pregnancy and neonatal outcomes data

Maternal data were obtained from the pregnant womens' medical notes at the Al Buraimi hospital where the childbirth occurred. These included pregnancy outcomes such as pre-eclampsia, GDM, gestational age at birth (weeks), type of childbirth (normal or CS), and preterm < 37 weeks or full term delivery > 37 weeks. Apgar scores were evaluated at 1 and 5 minutes after childbirth (65). Newborn anthropometric measurements were performed following standard techniques in the Ministry of Health in Oman by nurses at the delivery suite in the Al Buraimi Hospital. These included the length, weight, and head circumference of the infants. These measurements assessed the average birth weight or low birth weight (<2500 g) (74) and fetal macrosomia, defined as a newborn weight (≥ 4000 g) (539).

Table 2-2 Summary of experimental procedures conducted by the PhD student or collaborators:

Study	Description	Methods conducted by the PhD student	Conducted by others
Chapter 3: Depressive and anxiety symptoms and pregnancy outcomes among Omani pregnant women.	Estimate the prevalence of antenatal depression and anxiety symptoms among pregnant Omani women at weeks 8 – 12 weeks and 24 – 28 weeks of pregnancy in Omani pregnant women (N:302)	Consenting patients. An Arabic version of the self-administered Edinburgh Postnatal Depression Scale (EPDS) was used to measure anxiety and depressive symptoms in study participants.	
	Sociodemographic, maternal characteristics and pregnancy outcome data (N:302).	The study participants' sociodemographic and medical characteristics were documented using a general health and lifestyle questionnaire. The antenatal and pregnancy outcome data were obtained by reviewing patients' electronic medical records.	
Chapter 4: Maternal FAs intake and levels and the presence of depressive and anxiety symptoms. **And**	Fish and omega-3 fatty acids intake (N:302).	The estimation of the participants' fish and omega-3 fatty acid intake was measured using an altered version of the abbreviated FFQ of 7 questions. Data analysis	
	The assessment of the fatty acid profiles (proportions and absolute quantities) of maternal red blood cells (N=278) and cord blood samples (N=167).	Extraction of fatty acids from red blood cells. Running samples through gas chromatography GC Data analysis	Collection of blood samples from women in the study (staff nurse from the Antenatal Clinic at Al Buraimi Hospital).

Chapter 5: Fish and Omega Intake and FAs levels and pregnancy outcome.			Handling, processing and storage of red blood cells and plasma Layering down and centrifugation of whole blood for red blood cell and plasma isolation (Lab technician at Al Buraimi Hospital).
Chapter 6: Vitamin D, Vitamin B12 and Folate and prenatal depression and anxiety symptoms	The assessment of the vitamin D, vitamin B12 and folate blood test (N:302). Statistical analysis using SPSS software	Reviewing patients' electronic medical records for results of vitamin blood test Data entry, cleaning Conducting statistical tests	Collection of blood samples from women in the study (staff nurse from the Antenatal Clinic at Al Buraimi Hospital). Handling, processing, and storing red blood cells and plasma, layering down, centrifuging whole blood for red blood cell and plasma isolation and vitamin D, vitamin B12 and folate blood test (Lab technician at Al Buraimi Hospital).

2.6 Qualtrics survey

The sociodemographic and medical data of the pregnant women in the study were documented using a general health and lifestyle questionnaire, which was collected by an Internet-based survey created using Qualtrics software. The Arabic version of the self-administered (EPDS) scale and the 7-question abbreviated (FFQ) were also designed as Qualtrics surveys.

At the baseline assessment in the antenatal clinic, the pregnant women in Oman who chose to participate in the study were provided with an iPad tablet computer to complete the 3-part Qualtrics survey; the online survey included a general health and lifestyle questionnaire (EPDS) and the FFQ. Qualtrics software is an internet-based survey that allows researchers to conduct surveys with high levels of privacy for participants and store data online, making it easy to access at any time (540).

2.7 Anthropometric measurements

The anthropometric measurements for all sample of pregnant Omani women between 8-12 gestational weeks who participated in the study occurred at the antenatal clinic, Al Buraimi Hospital, and at the antenatal clinic, Primary Health Centre Al Buraimi Governorate .Body weight readings were recorded in kilograms with participants dressed in their clothing and standing with no shoes; therefore, a 0.5 kg subtraction was set into the device to account for the weight of the attire, and their height was measured to the closest 0.5 cm. Body weight was recorded by an adjusted electronic (Seca) scale precise to 1 kilogram. In addition, height was measured using a Seca 220 Telescopic Measuring Rod for Column Scales, accurate to 0.1 cm, with the pregnant women standing with no shoes. The standard method, WHO's BMI, was used to measure the body mass index

(BMI). This assesses weight divide up by the square of height (kg/m²) (541). BMI was used to classify pregnant women into three categories: average weight, overweight, and obese. WHO identifies a BMI of 19 -25 kg/m² as being the average weight, a BMI of 25-29.5 kg/m² as being overweight, and a BMI of 30 kg/m² or higher indicating obesity (542).

2.8 Measures of exposure

2.8.1 Seafood intake

The most common methods for measuring dietary intake in epidemiological research are 24-hour dietary records (24 Hr), food records (FR), and the Food Frequency Questionnaire (FFQ) (543).

The 3-day FR method is considered the gold-standard dietary assessment method in research settings for estimating macro- and micro-nutrient intakes such as carbohydrate, protein, and total fat intake (including n-3 FAS) (544). The FR method provides relatively accurate information on individual habitual dietary intake, including meals, eating frequency, and cooking methods: a food diary is no longer preferable in the research context because it is time-consuming and there is a high risk of bias (545). On the other hand, more recent attention has focused on the FFQ, which has been widely used in various studies of diet-disease interaction (546).

The FFQ is easy to administer, has a low participant burden, and less expensive than other dietary assessment tools. One advantage of the FFQ analysis tool is that it evaluates long-term diet, i.e., over several weeks to months, in contrast to 24 Hr dietary recall or the food record assessment method (546). Moreover, the FFQ can be designed to estimate the total nutrient intake of specific food items. Thus, it is a highly effective method for testing a research hypothesis (547). Furthermore, FA nutritional intake

assessed using dietary questionnaires such as FFQ has presented a strong correlation between studies in diet and disease associations (548).

Numerous validation studies have indicated that there is a strong association between dietary assessment methods, the FFQ, and the 3-day FR with other biomarker forms of validation, such as plasma composition and erythrocytes FAs content about omega-3 FAs intake (163,548–554). These studies conclude that the FFQ is reliable when it comes to estimating omega-3 FAs intake in epidemiological research (536,555–559). This applies to seafood constituents such as EPA and DHA omega-3 FAs, which are generally derived from diet intake (548,560).

The daily intake of EPA and DHA omega-3 FAs during pregnancy, primarily in the form of sea-food intake, is essential to meeting the enormous needs of the growing fetus in late pregnancy. Considering the critical role of DHA in pregnancy for the mother's health and cognition, visual acuity, and increased fetal weight (561), examining the daily consumption of omega-3 FAs during pregnancy is essential. Extensive research has shown that the FFQ could be the best dietary assessment tool in order to reflect the dietary intake of DHA and EPA during pregnancy, as it has shown high correlation coefficients and strong agreement (once validated) with plasma phospholipids (PL) and RBCs' FA levels (562–568).

There is a growing focus on measuring the total EPA and DHA intake in short and easy dietary intake tool formats such as electronic FFQs, such as that described by Swierk et al, (569) or by the brief, seven-question FFQ described by (536). Several validation studies have indicated that a short, targeted, and brief FFQ, explicitly designed to assess

the intake of EPA and DHA, is a reliable, low-burden dietary assessment tool that can improve research protocols (536).

In this research project, the participants' total dietary intake in terms of EPA and DHA was assessed using a modified version of the 7-question abbreviated FFQ designed by Kuratko et al, (536) to determine the daily intake in healthy adults. The author obtained permission to use and translate the abbreviated FFQ (Appendix 1).

The 7-question abbreviated FFQ was previously tested for its validity, reliability and reproducibility in small groups of healthy adults (67 participants): moderately significant correlation coefficients of DHA and EPA intake with two nutritional biomarkers - RBCs and plasma phospholipid - of the study sample were confirmed (536). Furthermore, the brief 7-question FFQ has been validated in a study sample of older adults and children in two different clinical trials: it shows significant correlation coefficients in terms of DHA intake with plasma phospholipids (570,571). However, several factors may influence the dietary intake assessment among pregnant women. For example, it is evident that when women become pregnant, they may try to eat healthier options to benefit their fetuses, which may not reflect their regular diets (572).

Pregnancy complications, especially gastrointestinal distress, including nausea and vomiting, also affect eating habits during pregnancy (573). Bed rest during pregnancy is another factor that could affect the dietary intake of pregnant women, as this may cause women not to cook or arrange foods as usual (572).

Moreover, DHA and EPA are only found in a few food sources that are not consumed frequently; this should be considered when measuring the dietary intake of pregnant women for research purposes (557).

A study were designed to assess the validation of the brief 7-question FFQ to estimate the DHA and EPA intake through the RBCs and plasma phospholipids (PL) in pregnant women (n=309) in their second trimester of pregnancy; the results indicated that the abbreviated FFQ was moderately associated with both EPA and DHA status (574).

The brief FFQ consisted of seven items. Each questionnaire item quantified portion size as a 3-ounce serving of food, according to DHA and EPA content. The brief FFQ has been modified to comply with portion sizes based on household serving units/utensils generally used in Oman with the aid of food models (162). The FFQ includes the frequency of intake of each food item/day, week, and month,

The primary purpose of the brief FFQ was linked to two direct measures: the type and frequency of oily fish and shellfish intake in the previous two months; and the participant's daily intake of DHA and EPA. The FFQ included measuring the frequency of food item intake per/day, /week, and /month and data on omega-3 FAs supplement intake. The first three questionnaire items seek information regarding fish and shellfish intake, categorised in terms of low, moderate and high EPA and DHA levels. Items four and five refer to liver and egg yolk intake; the sixth item covers chicken, turkey, and other forms of poultry intake. The seventh item collects information on omega-3 supplement intake.

The daily intake reference for each separate question was calculated according to average omega-3 (DHA, EPA) amounts of related foods in the nutritional analysis

software database FOODBASE version 3.1. This FOODBASE analysis program was previously validated by Doyle et al. (575,576). It was created based on enriched FAs reference data from the 5th edition of McCance and Widdowson's book The Composition of Foods. It was updated by the Institute of Brain Chemistry and Human Nutrition in London: dataset, plus its ten additional accompanying supplements (577).

Although small quantities of DHA and EPA can be found in red meats such as lamb and beef, significant amounts of such meats can add to the total intake of omega-3 DHA and EPA for those who do not prefer oily fish (578). Furthermore, the consumption of red meat in Oman is currently 2.5 times the consumption of fish (162). Conversely, omega-3 FAs may also be consumed from enriched or functional foods, which offer another nutritional source to consumers (579). Functional foods are recognised as supplemented or fortified foodstuffs that afford extra health benefits (580). Fortified foods containing omega-3 FAs include yoghurt, eggs, cereals, soup powder, juice, and plant oils (579). Even small quantities of these omega-3 FAs can turn into a significant level if consumed regularly in fortified foods (581).

Three questionnaire items were added to the original version to increase the brief FFQ's accuracy in estimating the daily omega-3 (DHA, EPA) intake among the study sample. The first item inquired about red meat consumption during the week, and the second item asked for information on fortified foods with omega-3; finally, the third item asked about vitamin and mineral supplement intake (mainly selenium, magnesium, zinc, folic acid, and vitamins B3, B6 and C), as they are used as co-factors to enhance the metabolism of omega-3 FAs (120).

2.8.2 Assessment of depressive symptoms

The depressive symptoms of women were measured using the Arabic version of the self-administered EPDS, translated by Mohammed et al (48). The EPDS is the most widely-recognised and commonly-used assessment scale for antenatal depression (582). The EPDS scale was designed and developed by Cox in 1987 to measure and screen postpartum depression in a community setting (583); it has also been successfully used and widely validated with a group of pregnant women (584). The author has received permission to use the Arabic version of the EPDS (Appendix 2).

An Arabic translation of the EPDS has previously been validated and successfully used among pregnant women in Oman (55,56), as well as in many Arab countries such as Morocco, Jordan, Tunisia, UAE, Bahrain, and Qatar, all of which have comparable sociodemographic and cultural features to Oman (48,50,52,54,582,585).

The EPDS rates the level of depressive symptoms in pregnant women during the previous week. It is intended to eliminate somatic symptoms of pregnancy, such as tiredness, sleeplessness, and weight gain. The scale remains easy to administer and has a 10-question psychometric rating scale. Every question is assessed on a 4-point scale from 0 to 3; the overall score varies from 0 to 30. Cox et al, suggested that an EPDS score of 10 is the cut-off point for measuring depressive symptoms among women in the postpartum period; however, it has been observed that a cut-off point of 13 or more has been achieved (95% of sensitivity and 93% of specificity) when likening the score with psychiatrists' assessment by using the "Diagnostic and Statistical Manual of Mental Disorders III (DSM III) criteria for depression" (586). It has also been shown that the

specificity and sensitivity of EPDS differs in terms of cultural concerns; as such, cut off points are different (587).

In this research project, the EPDS scores have been evaluated according to well-validated cut-off points for Arabic-speaking cultures (≥ 13) (48,588,589). Consequently, women who score between 0 and 9 are at little risk of experiencing antenatal depression symptoms. Women who score 10-12 will be at little risk of facing symptoms of prenatal depression (possible depression); scores of 13 or above indicate that a woman is experiencing symptoms of antenatal depression. However, the EPDS scale will not be used as a diagnostic tool, and careful clinical judgment will be used to confirm the diagnosis (590).

2.8.3 Assessment of anxiety symptoms

Anxiety during pregnancy is co-morbidly with prenatal and postnatal depression but often appears as an independent disorder (591). It is also linked with various detrimental pregnancy outcomes (592,593). Therefore, studies recommend that pregnant women undergo routine assessments for prenatal and postnatal anxiety (38).

The EPDS is recommended as a screening tool for assessing pre- and postpartum depression (582). It has been validated and interpreted in various languages and national settings (594). While it was initially designed to detect depression symptoms (583), several studies have suggested that EPDS can also act as a multidimensional tool for identifying anxiety symptoms among pregnant women (595). This recommendation is derived from the positive outcome of numerous factor analytic (EFA) studies that aim to assess the validation of a 3-item EPDS which contained three items of the 3A-EPDS scale item 3, item 4, and item 5 compared to various self-reporting anxiety tools (38,595).

Studies have identified a cut-off score of ≥ 6 for the 3A-EPDS that could be used to distinguish between pregnant women with different anxiety conditions. The evidence indicates that the 3A-EPDS with a cut-off score of ≥ 6 is recognised in about 70% of the women with known anxiety symptoms (595). It is essential to note that pregnant women recording high scores on the 3A-EPDS might not mark high on the total EPDS score and may not be identified if only the overall EPDS score is used (596).

The anxiety symptoms displayed by the pregnant women participating in the study were assessed using the Arabic version of the self-administered EPDS, translated by (48). The subscale 3A-EPDS with a cut-off score of ≥ 6 was used to assess the extent of anxiety disorder among the study sample. Considering the EPDS's prevalent worldwide practice as a measuring scale for pre- and postpartum depression, EPDS could similarly be used to consider maternal anxiety.

2.8.4 Repeat assessment of depression and anxiety symptoms

The second assessment point with regard to depressive and anxiety symptoms for the participants in the study took place between 24 and 28 gestational weeks at the antenatal clinic, AL Buraimi Hospital, and at the antenatal clinic, Primary Health Centre in the Al-Buraimi Governorate.

During this periodic review, the participants were given the Arabic version of the self-administered EPDS, translated by Mohammed et al, (48), to assess their depression and anxiety symptoms. As previously described, the EPDS scores of ≥ 13 were used to indicate a high level of experiencing symptoms of antenatal depression. As for the anxiety symptoms, a cut-off score of ≥ 6 in the subscale EPDS-3A was used to assess the extent of anxiety disorders among the study sample.

2.9 Laboratory assessment

2.9.1 Blood sample analysis

Fasting blood samples (5ml) were collected in a preserving tube containing ethylene diamine tetra acetic acid (BD Vacutainer® EDTA tubes) at 8-12 weeks of pregnancy. Within 4 hours of blood sample collection, samples were processed and separated into plasma layers, and erythrocytes were precipitated using icy (4°C) centrifugation at 1500 g for ten minutes. Once the plasma had been collected and stored at -40°C, the residual RBCs were washed three times in saline (0.9%) through centrifugation and then relocated into the additional tube for freezer storage. Erythrocyte membrane and plasma FAs were isolated and stored in the -80°C freezer until needed for analysis.

In this research project, the baseline erythrocyte and plasma FAs content were measured using blood samples taken from the women. It was kept at -80°C Celsius to extract the FAs from the blood samples using gas chromatography analysis. Due to the lack of facilities in Oman for omega-3 FAs analysis, all the collected erythrocytes were isolated and stored in the -80°C freezer in the medical lab of Buraimi Hospital without any further purification. They were then transferred to the Imperial College laboratories at Chelsea and Westminster Hospital in London.

The blood samples were shipped from the medical laboratory at Al Buraimi Hospital in Oman to the Imperial College London lab in London, UK, using dry ice in collaboration with the FedEx Logistics courier company. The shipment and packaging of the blood samples adhered to the Omani Customs' protocol for shipment of UN3373 dry ice samples. Blood sample collection, separation, and storage in Al Buraimi Hospital were supervised by the principal investigator (PI) (PhD candidate). The blood samples

analysed at Imperial College London were used only for research purposes and under the supervision of the PI. The PI only conducted data analyses. After the FAs analysis, the blood samples were disposed of according to Imperial College London and Chelsea and Westminster Hospital's clinical waste management facility regulations (ISS) (Appendix 3).

In this research project, a staff nurse from the Antenatal Clinic at Al Buraimi Hospital was required to take the blood samples from the participants in the study. Permission was obtained from the Al Buraimi Hospital Administration to use hospital resources for this purpose.

2.9.2 Umbilical cord blood

5 ml of cord blood samples were collected at childbirth in a preserving tube containing EDTA (BD Vacutainer® EDTA tubes). Within 4 hours of collection, the blood samples were processed and spilt into the precipitated erythrocytes and plasma layer through icy (4°C) centrifugation at 1500 g for ten minutes. The residual RBCs were washed three times in saline (0.9%) through centrifugation and then relocated into the additional tube for freezer storage erythrocyte's membrane (1 ml) in two tubes. Tube 1 was the original sample, and tube 2 was the duplicate sample, both of which were isolated and stored at -80°C until analysis.

2.9.3 Fatty acid extraction from erythrocytes

The concentrations of omega-3 FAs can be assessed by estimating the dietary intake, but it is more accurately measured by FAs analysis in the blood (597). Both FAs in RBC and plasma are effective biomarkers of omega-3 FAs (556,598). However, it is difficult to find a valid FA biomarker to reflect the actual omega-3 FAs intake as it can be affected

by endogenous metabolism, absorption, and hormones regulation (132,599–602). Erythrocytes membranes, also called RBCs, are considered a valid biomarker, reflecting the long-standing consumption of omega-3 FAs; plasma phospholipids indicate the omega-3 FAs intake in the previous month (132,603). A significant association links FA levels in the erythrocytes and other tissues, such as the brain. Hence it is preferable to analyse RBCs rather than plasma phospholipids (604).

The lipid extraction in this research project for maternal and cord erythrocytes samples was done following the Folch et al. approach (605). The maternal erythrocyte's aliquot part was defrosted using wet ice to avoid the breakdown of components and was gently doubled back 5 to 10 times to upset the concentration slopes shaped throughout thawing. The solvents used in the extraction process consist of butylated hydroxyl toluene (BHT).

In the lipids extract experiments, in 100 ml extraction tubes, 1 mL of erythrocytes sample was mixed with 15 mL methanol and 0.01 % BHT (Fisher Scientific). After that, 30 mL chloroform and 0.01 % BHT (100 mg/L) were added to the mixture in the 100 ml extraction tubes. Heptadecanoic acid (C17:0) (100 µL of 1 mg/mL), an internal standard, was added to the extraction tube holding RBCs mixed with C/M. The extraction tube was shaken carefully and capped instantly before storing it in a 4°C refrigerator for one day. Approximately 1 ml of RBCs was homogenised with 45 ml of C/M (2:1) + BHT.

The extraction tubes were removed from the refrigerator after 24 hours and allowed to warm up at room temperature for around 30 minutes to prevent condensation. The erythrocytes mixture was filtered into a partition funnel (conical metal) with grade 1 filter paper and washed with 10 ml + 5 ml of C/M (2:1) + BHT. The phase separation was then

completed by adding 25% of the 0.85% saline solution (Fisher Scientific). It was then kept at 4°C overnight.

The splitting funnels were removed from the refrigerator the following day and kept at room temperature for around an hour to allow them to achieve equilibrium. The organic layer was then placed in a 100 ml bottomed flask and evaporated with the aid of nitrogen gas. The mixture was then removed by a vacuum pump rotary evaporator (V-700, Buchi, Switzerland) using a water bath at a temperature of 37°C. The extracted lipid mixture was then placed in a 10 ml glass ampule. The bottle containing the lipid excess was then washed with 2 ml X 2 of dried methanol to eliminate any remaining moisture in the sample. It was cleaned three times with 2 ml of C/M and 2 ml of BHT and was stored at -20°C until further analysis.

Using GC, the methyl ester derivatives must be formulated with a volatile composition to determine the lipids' FAs profile. Methylation is a process commonly used by lipid analysts to transform rich acid content into methyl compounds. This method can increase the volatility of the FA, resulting in improved peak shapes in GC (606,607). The lipid elements were heated using a methylating agent containing 15% acetyl chloride in 100 ml of anhydrous methanol.

The methylating reagent was freshly made (less than 48 hours previously) by combining acetyl chloride gradually with cool and dried methanol. This process, which was performed at 70ºC for 3 hours, produced FA methyl esters (FAMEs). Methylating pipes were removed from the oven after 3 hours and chilled at room temperature for 10 to 20 minutes. After that, 2 ml of petroleum ether + (0.01%) BHT and 4 ml of sodium chloride solution (5%) were added up to the methylation tubes, stoppered tightly, and vibrated

vigorously to guarantee the maximum transmission of FAMEs into petroleum ether, resulting in two phases in each box. The upper phase was the FAME containing a petrol ether layer.

The upper layer of the methylation tube was then transferred to another vial containing 2 ml (2%) of potassium bicarbonate to counteract any acid that might have been added. This complete step was repeated twice in total but with 1 ml of petroleum ether + BHT (0.01%). The removal of FAMEs was performed with 4 ml of petroleum ether (2 ml + 1 ml). After vertexing, Tube 2 separated into two layers and contained potassium bicarbonate and petroleum ether. The FAMEs (upper layer) were then moved to a tube containing 100-200 grams of anhydrous sodium sulphate to remove any residual water. Finally, the FAMEs mixture was transferred to a marked 3 ml glass vial. Subsequently, the FAMEs were dried using a nitrogen gas stream, mixed with 1 mL of heptane (0.01% BHT), and stored at -20°C until GC analysis.

The separation was explicitly devised for FAs methyl ester (FAME) analysis. After extracting the FAs, the FAMEs were separated using GC. The separated compounds were then loaded into an autosampler ampule then repeatedly injected into the Agilent GC-7890A device equipped with an MS-Premium, low bleed column (60 m x 0.32 mm ID, 0.25 µm film, BP20) (SGE Analytical Science, Australia) range with a maximum temperature up to 260°C. The carrier gas employed was hydrogen, and FAMEs were detected by evaluating their retention times to an accurate standard, the 37-component FAME mix (Sigma Aldrich). Made from lipid extracts of olive oils (including stearidonic acids, gamma-linolenic and alpha-linolenic acid). The modules of the FAMEs mix's

accurate standards can be seen in Table 2.3 below. Specifically, retention times were used to detect the FAMEs.

2.9.4 Optimisation of GC internal standard

The GC internal standard (IS) principle enables the measurable chemical analysis of FAMEs. The (IS) is designed to add a certain quantity of IS to every sample to adjust the reaction between the (IS) and the analyte. The advantage of using the (IS) is its ability to equal the analyte's chemistry at every step of the process, including adjusting for losses. For studies that examine the erythrocyte's FA content, the usual standard for analysis is heptadecanoic acid (608–611).

2.9.5 Gas Chromatography (GC)

Critical chemistry analysis using GC is performed in order to separate and assess mixtures that have been vaporised without thermal decomposition (612). This process is commonly utilised for regulating the relation focuses of FAs in food and biological samples. (613) first suggested that GS can be used as an analytic tool in a wide range of sciences. Later, in 1951, James and Martin established their theory explaining the workings of the first gas chromatograph used in analysing volatile FAs (614). The separation of explosive organic mixtures using GC depends on the stationary and mobile phases' unique characteristics. The inert gas GC comprises a flowing mobile step and an injection point. It has a column containing components of a data recording system and a detector. The sample is set in the injection port in the mobile phase before its move to the stationary phase. The element’s separation is detected at different retention times depending on the time needed to travel through the column. The data recording system

is connected to the detector and is used to determine the FAs present in a sample. The areas below the chromatographic peaks are related to the volume of FAs in the model.

2.9.6 GC data analysis

Table 2-3 The FAME's standard contains 37 components:

37-Component FAMEs Standard
Methyl butyrate 400 µg/mL
Methyl hexanoate 400 µg/mL
Methyl octanoate 400 µg/mL
Methyl decanoate 400 µg/mL
Methyl undecanoate 200 µg/mL
Methyl laurate 400 µg/mL
Methyl tridecanoate 200 µg/mL
Methyl myristate 400 µg/mL
Methyl myristoleate 200 µg/mL
Methyl pentadecanoate 200 µg/mL
Methyl *cis*-10-pentadecenoate 200 µg/mL
Methyl palmitate 600 µg/mL
Methyl palmitoleate 200 µg/mL
Methyl heptadecanoate 200 µg/mL
cis-10-Heptadecanoic acid methyl ester 200 µg/mL
Methyl stearate 400 µg/mL
trans-9-Elaidic acid methyl ester 200 µg/mL
cis-9-Oleic acid methyl ester 400 µg/mL
Methyl linolelaidate 200 µg/mL
Methyl linoleate 200 µg/mL
Methyl arachidate 400 µg/mL
Methyl γ-linolenate 200 µg/mL
Methyl *cis*-11-eicosenoate ≤ 200 µg/mL
Methyl linolenate 200 µg/mL
Methyl heneicosanoate 200 µg/mL
cis-11,14-Eicosadienoic acid methyl ester 200 µg/mL
Methyl behenate 400 µg/mL
cis-8,11,14-Eicosatrienoic acid methyl ester 200 µg/mL
Methyl erucate 200 µg/mL
cis-11,14,17-Eicosatrienoic acid methyl ester 200 µg/mL
cis-5,8,11,14-Eicosatetraenoic acid methyl ester 200 µg/mL
Methyl tricosanoate 200 µg/mL
cis-13,16-Docosadienoic acid methyl ester 200 µg/mL
Methyl lignocerate 400 µg/mL
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester 200 µg/mL
Methyl nervonate 200 µg/mL
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester 200 µg/mL

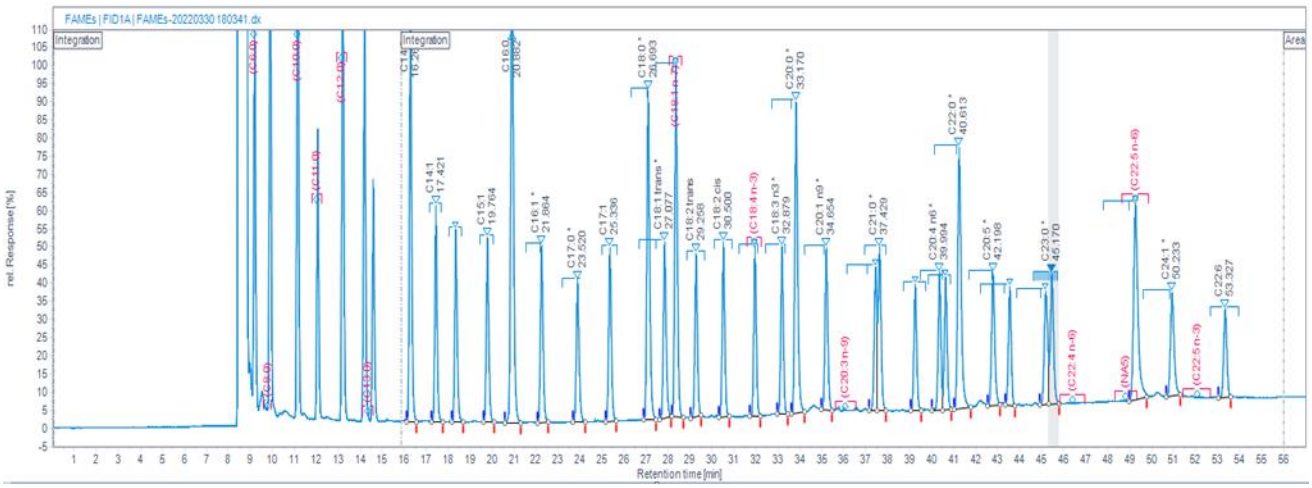

Figure 2-2 A sample chromatogram demonstrating the presence of fatty acids compared to authentic standards.

Analyte peaks were each assessed compared to the FAMEs, and double estimations were made based on the chromatographic analysis of the sample, namely, (1) the absolute quantity and (2) the % of total FAs. A computer-based data chromatography system was utilised to determine the peak areas in the sample in terms of percentage values (Agilent Open LAB chromatography data system, Scientific Software Inc., San Ramon, CA). The peak areas were calculated based on the percentage of total peaks identified. The database only created the absolute size of each peak for total quantification, and the last measures had to be determined manually using the equation below.

The amount of FA per 1 ml of an erythrocyte aliquot is determined by the FAME's peak and volume of internal standard (IS) peak and volume. Individual FA (mg) per 1 mL of erythrocytes aliquot, if 1µL is comparable to 1µg: FAMEs peak and the amount of IS (µL

(ml) x standard internal height x 1000. Different terminologies were used in this thesis to identify FA analysis data (Table 2-4).

Table 2-4 The terms used in the thesis to analyse fatty acids:

Term	Classification	Unit
Fatty Acids status	Fatty acids content measured in RBCs	Percentage (%) of total fatty acids
Fatty Acids Levels/concentration percentages/proportions	The individual fatty acid level in relation to all fatty acids in 1 ml of RBCs	Percentage (%) of total fatty acids
Fatty acid absolute quantities/levels	The individual fatty acid quantity/1 ml of RBCs	mg/g

The analysis of RBCs and plasma FAs content in the GC include: mono-unsaturated FAs (c14:1 to c24:1), saturated FAs (c14:0 to c24:0), total omega-6 FAs, docosapentaenoic acid (omega-6 22:5); docosatetraenoic acid (omeg-6 22:4); AA (omeg-6 20:4); eicosatrienoic acid (omeg-6 20:3); eicosadienoic acid (omeg-6 20:2); γ linolenic acid (omeg-6 18:3); [linoleic acid (omeg-6 18:2)]. Total omega-3 FAs, DHA (omeg-3 22:6); EPA (omeg-3 20:5); α-linolenic acid (omeg-3 18:3); docosapentaenoic acid (omeg-3 22:5), AA/LA Osbond acid/adrenic acid (AdA) = 22:5/22:4ω6, the (AA)/DHA, omega-3 index, (AA+DHA)/MUFA omega-6/omega-3 ratio was also included in the analysis outcome.

2.9.7 Vitamin D analysis

Due to its stability within the human body and its role as the primary circulating form of vitamin D (615), 1,25-Dihydroxyvitamin D (DVHD) is measured as the dynamic component of vitamin D. However, the concentration of DVHD does not reflect the body's actual level of vitamin D. Instead, several researchers have used vitamin D3 in order to examine the vitamin D levels in patients (345,467,616), Therefore, in this study, serum

total vitamin D3 (25[OH]D3) analyses were used to verify the vitamin D levels of the study participants.

Blood samples (5 ml) were collected from Omani pregnant women on a separating tube and sent to Al Buraimi Hospital Laboratory for further analysis. A maternal venous blood sample was collected at study enrolment at 8–12 weeks (n=298). The blood sample was left to clot, centrifuged, and stored at about –40º C. Maternal 25(OH)D3 levels were assessed on the same day for every 100-blood samples. A serum total (vitamin D3) was evaluated using the Cobas-e 411 immunoanalyser with a reasonable electrochemiluminescence (ECL) protein binding assay and various reagents acquired from Roche Diagnostics. The calibration ranged from 3 to 70 ng/ml (617). The coefficients of variability for the intra- and inter-assay tests were 2.8–5.1% and 1.5–4.6%, respectively.

The vitamin D analysis was done in Oman's Al Buraimi Hospital laboratory. The vitamin D status was classified as follows: (a) normal range: serum (vitamin D3) concentration (> 50 nmol/L) (< 30 nmol/L), (b) insufficient range: serum (vitamin D3) levels between (31-50 nmol/L), and (c) deficient range: serum (vitamin D3) levels.

2.9.8 Folate and vitamin B12 analysis

To measure the vitamin B12 and folate status, maternal blood samples (5ml) were collected from Omani women at the 8^{th}–12^{th} week of pregnancy (enrolment stage for the study). Blood samples were collected using regular, red-topped vacutainers with no anticoagulant. Within 4 hours of collection, the blood samples were subjected to a centrifuge for around 10 minutes at 4°C. They were then isolated at −20°C and liquefied before investigation.

Tests of serum vitamin and B12 folate levels were performed at the Al Buraimi Hospital laboratory using Roche reagents electrochemiluminescence in a mechanical Cobas Team 411 (Roche). The direct sequence of the assay for folates is 1.6 to 20.0 ng/mL, CVs of 4% at 3.9 ng/mL and 3.2% at 17.6 ng/mL; The assay sequence for vitamin B12 is 84 to 2000 pg/mL, with CVs of 7% at 246 pg/mL and 5% at 890 pg/mL. Both plasma vitamin B12 and folate measurements were conducted within international standards recognised for the certified method agreed upon by the Ministry of Health in Oman for such blood tests.

2.10 Potential confounders

Information on sociodemographic variables included other factors that are potential confounders with seafood intake or depression; these were recorded and used as adjustment variables in the data analysis. Confounders included maternal age, parity, marital status, number and outcome(s) of previous pregnancies, highest educational qualification achieved, housing tenure, occupational status, and annual household income.

Information also included various lifestyle and health data: maternal smoking (no/yes); if yes, how many cigarettes smoked/day (prior to and throughout pregnancy); pre-pregnancy and current BMI; and the exercise levels of the participants. Data collected also included information about neonatal outcomes taken from Child Health Records (such as gestational weeks at birth, recumbent birth length, birth weight, Apgar score, and the date and sex of birth. The sociodemographic and medical data of the pregnant women who participated in the study were recorded using the general health and lifestyle questionnaire (Appendix 5).

2.11 Statistical analysis

Descriptive statistics were used to outline the pregnant women's demographic, socioeconomic, and psychological variables at the three assessment points of the study. This included fish intake and omega-3 FAs intake from fish on the part of the participants in the study. The mean ± standard deviation was used for normally distributed variables, whereas the median range was utilised for variables not normally distributed. Frequencies and percentages were considered for categorical variables. The Kolmogorov-Smirnov statistics were used to confirm the normality of the data. Three categories of fish consumption were created :(a) Never; (b) 1-2/week; (c) > 2/week to evaluate the relationship between prenatal depression and anxiety symptoms in pregnant women and fish intake. The fish intake categories are in line with the international recommendations about fish intake during pregnancy in Europe and the UK (159,191,193), which advise pregnant and lactating women to increase their fish intake to ranges between 1-2 up to 3–4 fish portions/week.

The relationship between fish and EPA and DHA omega-3 FAs intake and level with anxiety and depression symptoms and the association between FA levels and pregnancy outcomes among the study samples were assessed. For categorical variables, the Chi-squared test was used, while for continuous variables, the Mann-Whitney U test was employed. A series of logistic regression modules using the remove method of multiple regression methods were applied to check the effect of potential confounders and FAs levels, fish intake, DHA, and EPA intake variables on the incidence of anxiety and depression symptoms. The logistic regression analysis tests were set to evaluate 95% confidence intervals (CIs) and odds ratios (ORs). Both tests were used to explore the

relationship with anxiety symptoms (EPDS-3A score <6 v. EPDS-3A score ≥6), depressive symptoms (EPDS score <13 v. EPDS score ≥13), and pregnancy outcomes. The depression and anxiety symptoms score, fish + EPA, and DHA intake variables were entered in the first step; the second step involved joining the sociodemographic variables and, finally, the health and lifestyle variables.

The operative confounding of depression and anxiety and FA levels and the fish and omega-3 FAs intake was assessed using a sequence of regression statistics tests in which the uncontrolled regression coefficient was linked with that found after the outline of a potential confounder. The potentially confounding variables included maternal age, highest educational level, marital status, parity, history of depression or anxiety, previous pregnancies, employment status, monthly family income, physical activity, and BMI. Any variable that originated from being significantly linked with high depression and anxiety rate during pregnancy, or with fish intake and DHA and EPA intake or its occurrence, produced a coefficient to variation by above 10% was taken to be a confounder, and engaged in subsequent multivariable analysis.

2.12 Ethical considerations

The ethical approval application was submitted to the RERAC in the Ministry of Health in Oman on 17th March 2019. On the 1st of July 2019, the Board of the Research Committee in the Ministry of Health in Oman approved the study protocol (see Appendix 4). Following the Institutional Review Board's approval, all pregnant women in the study were asked to provide written informed consent. Participants were recruited with the regional health authorities and with the assistance of the antenatal clinics at Al Buraimi Hospital and the Polyclinic Centre in Al Buraimi.

Pregnant women were given the Participant Information Sheet (PIS) (see Appendix 6) to review in the reception area before they consulted with the obstetrician as part of the routine antenatal clinic check-up. During the consultation, the obstetrician asked the eligible women if they were concerned about participating in the research. The participants who agreed to participate were referred to the PI, who explained to them the importance and expected results of the study. The PI described the stages of completion of the survey using the PIS. The pregnant women had enough time to read and understand the PIS. They had the right to ask about any part of their participation in the study before they made an informed decision or gave their consent (see Appendix 7) to be involved in the study.

Next, the pregnant women were given the EPDS (Arabic version) to assess their depressive symptoms score and the semi-quantitative FFQ to assess their level of seafood and omega-3 FAs intake. They were also given a general health and lifestyle questionnaire to collect sociodemographic data and other medical characteristics. Any missing demographic data, or additional necessary information, was collected from the patient's medical notes by the PI, who had been granted authorisation to access the patient's medical notes as he is a staff member of Al Buraimi Hospital. The subjects needed to be available for the entire length of the study and be fully prepared to meet all the study requirements, all of which were explained clearly before recruitment and consent had been given. The participants were made aware that they could withdraw from the study and that their data could be extracted at any stage without providing a reason. As legitimate Omani patients, their withdrawal would not jeopardise the treatment they were to receive from the hospital. An exit interview was conducted, during which

participants were asked to rank their compliance from 0 to 10 and give reasons for non-compliance. Any adverse events were recorded at this stage.

Chapter 3: Depressive and anxiety symptoms and pregnancy outcomes among Omani pregnant women

3.1 Introduction

Maternal depression during and after pregnancy are a worldwide public concern (29,618). Maternal depression negatively affects health status of mothers and child development (619). It is one of the several types of disability among pregnant women globally, mainly in low-income populations (19). While the incidence of antenatal depression differs between countries, it is usually more prevalent than postpartum depression (48,620).

The incidence of prenatal depression among women is 15.6% worldwide (621). The progress of depressive symptoms increases significantly throughout pregnancy (622) and is expected to occur in the middle of pregnancy (623). Meta-analysis research aiming to estimate the incidence of antenatal depression in developed countries indicates that 12% of pregnant women suffered depression in the third trimester, 12.8% (10.7%–14.8%) in the second trimester and 7.4% (2.2%–12.6%) in the first trimester (18).

An increasing level of pregnancy-related anxiety and depressive symptoms has been documented in Oman, both in clinical settings and in an epidemiological survey conducted in the community (55). The authors reported that 12% of pregnant women in Oman are at risk of developing postnatal depression. In a recent observational study involving 959 pregnant Omani women (56), 24.3% of the study sample suffered from antenatal depression. This is considered high, particularly in comparison with findings from other Arab countries (29,58).

Antenatal depression has various risk factors, including educational levels, sociodemographic and socioeconomic status, low social support, marital disputes, physical violence from a partner and a history of depression and anxiety (35,48,624). The development of prenatal depression is significantly impacted by biological and medical risk factors (625,626). In a recent study, marital conflict and unplanned pregnancy were substantial predictors of maternal depression in Oman (56).

Antenatal depression is associated with PE, caesarean delivery (CS), suicidal ideation, and a significant risk of postnatal depression (23,627). Several studies have indicated a strong link between antenatal depression with LBW, preterm delivery, high admission rates to special care baby units, fetal death, and poor child development (27,627,628).

Antenatal depression is frequently misdiagnosed and not treated, leading to poor pregnancy outcomes (620). Therefore, improved awareness and routine evaluation of depression symptoms during pregnancy with appropriate mental health interventions will significantly improve the mothers' and children's health status (628,629).

Depression symptoms during pregnancy could result from anxiety or failure to cope with stress (24). Anxiety is generally co-morbid with depression but is commonly not discussed in psychiatric research on pregnant and lactating women (630). Furthermore, anxiety disorders are likely to affect approximately 15% to 23% of pregnant women and are associated with a high rate of a range of different adverse consequences for the mother and child (24,40,42).

Prenatal anxiety may increase the risk factor for poor child development (631). Maternal anxiety is a significant predictor of postpartum anxiety (35,632,633). It is associated with

increased concern about childbirth (634), preterm birth (635), CS delivery (636), and suicide attempts in severe cases (637). It has also been associated with poor Apgar scores (638).

3.2 Aim

1- To estimate the prevalence of antenatal depression and anxiety symptoms among sample of pregnant Omani women at weeks 8 – 12 weeks and 24 – 28 weeks of pregnancy in Omani pregnant women.

2- To identify the risk factors associated with antenatal depression and anxiety symptoms among Omani pregnant women.

3- To identify the association between antenatal depression and anxiety symptoms with maternal and neonatal outcomes among Omani pregnant women.

3.3 Methods

This cohort study recruited 300 pregnant Omani women receiving maternal care at Al Buraimi Hospital, Oman. Participants were recruited from the antenatal clinics of Al Buraimi Hospital and the Primary Health Center in Al Buraimi, which serves the local community of Al Buraimi Governorate. The methods used, including inclusion and exclusion criteria, are described in detail in Chapter 2. The Ethics and Research Committee of the Ministry of Health of Oman approved the study protocol on 1 July 2019 (MoH/CSR/19/9668) (The ethical approval form is attached in Appendix 4).

After meeting the inclusion and exclusion criteria and following the obtaining of fully informed written consent, the anxiety and depressive symptoms in the study participants were measured using the Arabic version of the self-administered Edinburgh Postnatal

Depression Scale (EPDS) as translated by Mohammed et al, (48). For this study, permission to use the Arabic version of the EPDS was obtained (Appendix 2). The Arabic translation of EPDS has previously been validated and successfully used among pregnant women in Oman (55,56), as well as in many Arab countries such as Morocco, Jordan, Tunisia, UAE, Bahrain, and Qatar, all of which have sociodemographic and cultural characteristics comparable to Oman (48,50,52,54,582,585). In this study, EPDS with a cutoff score of 13 was used to assess depression symptoms and the subscale (EPDS-3A) with a cutoff score of (≥ 6) was used to assess anxiety symptoms among the study sample (595).

The information on sociodemographic variables includes other factors that are potential confounders of depression; These were recorded and used as adjustment variables in the data analysis. The sociodemographic and medical characteristics of the study participants were recorded using the general health and lifestyle questionnaire (Appendix 5). In summary, patient electronic medical records were reviewed to obtain antenatal and intrapartum data. Maternal data was obtained from medical records of pregnant women at the Al Buraimi hospital where the birth occurred. These include pregnancy outcomes such as PE, GDM, and mode of delivery (normal or CS), gestational age at delivery (weeks) and preterm < 37 weeks or full-term delivery > 37 weeks, according to WHO standards (65). The anthropometric measurements of the newborns were performed following standard techniques in the Oman Ministry of Health by nurses in a delivery suite at AL Buraimi Hospital. These included the length, weight, and head circumference of the infants at birth, and assessed low weight (LBW <2500 g) (74), fetal macrosomia (weight >4000 g) (539), and Apgar scores at 1 minute and 5 minutes.

3.3.1 Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 21, developed by IBM Corp., Chicago, Illinois, USA. Descriptive statistics were used to describe the socioeconomic, demographic, and psychological data of pregnant women. The results were expressed as means and standard deviations (SD) for continuous variables, while categorical variables were presented as frequencies and percentages.

Histograms and box plots were produced with respect to continuous variables to study the distribution and identify outliers. To investigate the link between antenatal depression and anxiety symptoms with sociodemographic factors, we conducted a univariate analysis using Pearson's chi-square test. A p-value of ≤0.05 was deemed statistically significant.

Logistic regression was employed to analyses odds ratios (OR and 95% confidence intervals (CI) to assess statistical significance. Both tests were used to explore the relationship with anxiety symptoms (EPDS-3A score <6 v. EPDS-3A score ≥6), depressive symptoms (EPDS score <13 vs. EPDS score ≥13), and pregnancy outcomes.

To adjust for potentially confounding influences, additional analysis was performed using multiple regression statistics tests for variables that showed substantial links with depression and anxiety symptoms at the $p \leq 0.05$. The entry method and the removal regression method were used for the analysis. All variables were entered in the multiple logistic regression model, and those with the least contribution to overall variance were eliminated. Potential confounder variables included maternal age, parity, marital status,

annual household income, history of depression, anxiety disorder, physical activity, annual household income, employment status, educational level, and pre-pregnancy BMI.

3.4 Results

3.4.1 Sociodemographic and Clinical Characteristics

Table 3-1 shows the sociodemographic and gestational characteristics of the sample of pregnant Omani women involved in the study. In total, 302 pregnant Omani women agreed to participate in this cohort study. The mean age was 30.8 years (SD 6.103, range 28 years. Most of the study samples were from the age group between 20 and 30 years (44.7%) and between 30 and 40 years (46.4%). Approximately 47% of the study sample had a university degree or higher, and 44% of the pregnant women had completed secondary school. Most of the study sample (70%) were homemakers, while 30% were employed. About 40% had a monthly family income of 500-1000 OMR, and 45% of the study sample had a monthly family income of less than 500 OMR per month. In terms of type of accommodation, 26% of pregnant women lived in rented housing, 39% lived in their own house, and 35% lived in the home of the husband or wife's family. About 19% of the women reported having been married for less than two years, 20% for more than 2 years but less than five years, and 35% for more than 5 years but less than ten years. 67% of the participants had planned their pregnancy. Furthermore, 8% reported having a family history of depression and 10% of the study sample reported having a history of depression. More than half of the study sample (53%) did not exercise, while 28% reported exercising for 30 minutes at least twice a week. Women in the study sample were between 8 and 12 weeks of gestation at the time of recruitment. In most of the

sample, 67% did not report medical complications in previous pregnancies, while 25% had a history of miscarriage. In terms of mode of delivery in previous pregnancies, 54% of the study sample had normal delivery (SVD), and 28% had CS. At baseline measurements (at 8 -12 weeks of pregnancy), 29% of pregnant women were overweight (25-29.5 kg/m²), and 39% were obese (30 kg/m² or higher). Regarding parity, 23% were nulliparous, 24% were primiparous, and 53% were multiparous.

At the end of the study, 242 pregnant Omani women completed the three assessment points, including pregnancy and neonatal outcomes (Table 3-1). Of the group that completed the study, most were between 30 and 40 years old (48.8%), 47% of the study sample had a university degree or higher, s 69% were homemakers, 30% were employed, 24% lived in rented housing, and (37%) live with a husband or wife family. In terms of type of accommodation, 24% of pregnant women lived in rented housing, 38% lived in their own house, while 37% lived in the home of the husband or wife's family.

Around 69% of the participants had no medical complications during previous pregnancies. At the same time, 24% had a history of miscarriage, and 4% had preterm delivery. Among all women in the study, 18.6 % of pregnant women reported that their duration of marriage is less than two years, and 67 % of the women in the study have planned for their pregnancy. Approximately only 9.5 % reported having a previous family history of depression, and 11.6 % of the study sample reported having a history of depression. More than half of the study sample (55%) reported not participating in physical activity, about 43.4% had adequate gestational weight gain GWG, and 49.6% had low GWG. In terms of mode of delivery in previous pregnancies, 53 % of the study sample had normal delivery (SVD), and 30 % had CS.

Table 3-1 Sociodemographic and gestational characteristics of pregnant Omani women:

Variables	8 – 12 weeks of pregnancy		24 – 28 weeks of pregnancy		At child birth	
	N (302)	%	N (272)	%	N (242)	%
Age (Y)						
< 20	9	3	8	2.9	5	2.1
20 – 30	135	44.7	121	44.5	103	42.6
30 – 40	140	46.4	126	46.3	118	48.8
> 40	18	6	17	6.3	16	6.6
Educational level						
Basic Education	28	9.3	25	9.2	25	10.3
Secondary Education	133	44.0	120	44.1	103	42.6
Degree or higher	141	46.7	127	46.7	114	47.1
Occupational Status						
Employed	93	30.3	82	30.1	73	30.2
Unemployed	209	69.7	190	69.9	169	69.8
Annual household income						
Less than 500 OR	134	44.4	121	44.5	109	45
500 OR – 1000 OR	122	40.4	109	40.1	94	38.8
Above 1000	46	15.2	42	15.4	39	16.1
Housing tenure						
Rent house	78	25.8	70	25.7	59	24.4
Homeowner	117	38.7	103	37.9	93	38.4
Live with the husband or wife's family	107	35.4	99	36.4	90	37.2
Duration of Marriage						
Less than 2 years	57	18.9	52	19.1	45	18.6
Less than 5 years	61	20.2	59	21.7	47	19.4
Less than 10 years	108	35.8	96	35.3	88	36.4
Above than 10 years	76	25.2	65	23.9	62	25.6
Planned pregnancy						
Yes	202	66.9	178	65.4	162	66.9

No	100	33.1	94	34.6	80	33.1
Physical Activity						
No	158	52.3	143	52.6	132	54.5
30 minutes for at least 2/ week	86	28.5	76	27.9	65	26.9
30 minutes for at least 3/week	28	9.3	24	8.8	23	9.5
30 minutes for at least 5/week	30	9.9	29	10.7	22	9.1
History of Depression						
Yes	32	10.6	30	11.0	28	11.6
No	270	89.4	242	89.0	214	88.4
Family history of depression						
Yes	26	8.6	24	8.8	23	9.5
No	276	91.4	248	91.2	219	90.5
Parity						
0	69	22.8	63	23.2	54	22.3
1	72	23.8	64	23.5	56	23.1
2+	161	53.3	145	53.3	132	54.5
BMI at registration						
Underweight (less than19 kg/m²)	23	7.6	21	7.7	16	6.6
Normal (19-25 kg/m²)	75	24.8	67	24.6	54	22.3
Overweight (25-29.5 kg/m²)	86	28.5	80	29.4	78	32.2
Obesity (30 kg/m² or higher)	118	39.1	104	38.2	94	38.8
Obstetric complication Previous Pregnancy						
Null	203	67.2	188	69.1	58	24
Preeclampsia (PE)	8	2.6	64	23.5	7	2.9
Miscarriage	76	25.2	8	2.9	10	4.1
Preterm	15	5	12	4.4	167	69
Mode of delivery in previous pregnancies						
Normal Delivery (SVD)	164	54.3	145	53.3	130	53.7
Caesarean section (CS)	86	28.5	79	29.0	73	30.2
No previous pregnancies	52	17.2	48	17.6	39	16.1

3.4.2 Prevalence of antenatal depression

Table 3-2 shows the relationship between prenatal depression and sociodemographic factors. EPDS scores ranged from 0 to 25 (mean: 10.34). Table 3-2 shows that the incidence of prenatal depression was 29.8% at 8 – 12 weeks and 30.5% at 24 – 28 weeks, using a cutoff point of -13 on the EPDS scale.

In Table 3-2, the univariate analysis showed a significant association linking prenatal depression at 8-12 weeks of pregnancy and the type of living arrangement. This is particularly evident in pregnant women who resided with their spouse's family (P = 0.021), have a history of depression (P = 0.001), or have a family history of depression (P = 0.04).

At the same time, in the univariate analysis, antenatal depression at 24 to 28 weeks of gestation was significantly associated with pregnant women who had completed university education (P = 0.028) and who had a history of depression (P = 0.000). None of the following variables: maternal age, occupational status, monthly family income, duration of marriage, parity, previous pregnancy, obstetric complications, mode of delivery in previous pregnancies, or BMI at registration that were included in the bivariate analysis were significantly related to prenatal depression among women participating in the study.

Table 3-2 Associations between antenatal depression and variables of sociodemographic and gestational characteristics among pregnant Omani women:

	8 – 12 weeks of pregnancy N = 302						24 – 28 weeks of pregnancy N = 272					
Variables	**Non- depressive (EPDS ≤12) (n=212) (70.2%)**		**Depressive (EPDS) ≥ 13 (n=90) (29.8%)**				**Non- depressive (EPDS ≤12) n=189 (69.5%)**		**Depressive (EPDS) ≥ 13 n=83 (30.5%)**			
	N	%	N	%	(*df*) χ^2	*p*	N	%	N	%	(*df*) χ^2	*p*
Age (Y)					0.606	0.891					1.568	0.664
< 20	6	2.8	3	3.3			5	2.6	3	3.6		
20 – 30	95	44.8	40	44.4			83	43.9	38	45.8		
30 – 40	97	45.8	43	47.8			87	43.9	39	47		
> 40	14	6.6	4	4.4			14	7.4	3	3.6		
Educational level					3.59	0.163					7.08	***0.028***
Basic Education	24	11.3	4	4.4			22	11.6	3	3.6		
Secondary Education	92	43.4	41	45.6			87	46	33	39.8		
Degree or higher	96	45.3	45	50			80	42.3	47	56.6		
Occupational Status					1.970	0.166					0.337	0.567
Employed	69	32.5	22	24.4			59	31.2	23	27.7		
Unemployed	143	67.5	68	75.6			130	68.8	60	72.3		
Annual household Income					3.224	0.195					2.00	0.367
Less than 500 OR	89	42	45	50			86	45.5	35	42.2		
500 OR – 1000 OR	86	40.6	36	40			71	37.6	38	45.8		
Above 1000	37	17.5	9	10			32	16.9	10	12		
Housing tenure					7.723	***0.0213***					4.105	0.128
Rent house	54	25.5	24	26.7			45	23.8	25	30.1		
Homeowner	92	43.4	25	27.8			79	41.8	24	28.9		
Live with the husband or wife family	66	31.1	41	45.6			65	34.4	34	41		
Duration of Marriage					0.926	0.819					7.646	0.054
Less than 2 years	41	19.3	16	17.8			38	20.1	14	16.9		
Less than 5 years	42	19.8	19	21.1			37	19.6	22	26.5		

Less than 10 years	73	34.4	35	38.9			53	28	12	14.5		
Older than 10 years	56	26.4	20	22.2			53	28	12	14.5		
Planned pregnancy					1.931	*0.165*					3.059	0.080
Yes	147	69.3	55	61.1			130	68.8	48	57.8		
No	65	30.7	35	38.9			59	31.2	35	42.2		
Physical Activity					1.598	*0.660*					4.793	*0.188*
No	108	50.9	50	55.6			97	51.3	46	55.4		
30 minutes for at least 2/ week	64	30.2	22	24.4			56	29.6	20	24.1		
30 minutes for at least 3/week	18	8.5	10	11.1			13	6.9	20	24.1		
30 minutes for at least 5/week	22	10.4	8	8.9			23	6.9	11	13.3		
History of Depression					21.957	***0.001***					13.826	***0.001***
Yes	11	5	21	24			12	6.3	18	21.7		
No	201	95	69	76			177	93.7	65	78.3		
Family history of depression					3.636	***0.04***					0.606	0.436
Yes	14	6.6	12	13.3			15	7.9	9	10.8		
No	198	93.4	78	86.7			174	92.1	74	89.2		
Parity					0.693	*0.707*					1.164	0.559
1	49	23.1	20	22.2			45	23.8	18	21.7		
2	53	25	19	21.1			41	21.7	23	27.7		
3	110	51.9	51	56.7			103	54.5	42	50.6		
BMI at registration					1.812	0.612					3.112	0.375
Underweight	14	6.6	9	10			15	7.9	6	7.2		
Normal	55	25.9	20	22.2			48	25.4	19	22.9		
Overweight	58	27.4	28	31.1			60	31.7	20	24.1		
Obesity	85	40.1	33	36.7			66	34.9	38	45.8		
Obstetric complication Previous Pregnancy					4.179	0.243					2.168	0.538
Preeclampsia (PE)	8	3.8	0	0			7	3.7	1	1.2		

Miscarriage	50	23.6	26	28.9			46	24.3	18	21.7		
Preterm	11	5.2	4	4.4			7	3.7	5	6		
Mode of delivery in previous pregnancies					1.723	0.423					1.220	0.543
Normal Delivery (SVD)	110	51.9	54	60			97	51.3	48	57.8		
Caesarean section (CS)	63	29.7	23	25.6			56	29.6	23	27.7		
No previous pregnancies	39	18.4	13	14.4			36	19	12	14.5		

Table 3-3 shows the outcomes of the logistic regression for the predictors of antenatal depression using the odds ratio (95% CI) among the study sample. The study revealed that the level of education of pregnant women had a significant association with antenatal depression in that the chances of depression were 6.5 times higher among women who had completed university education between 8 and 12 weeks of pregnancy [AOR=6.55; 95% CI (1.70-25.13)] and 8.1 times at 24 – 28 weeks of pregnancy [AOR=8.13; 95% CI (1.84-35.82)]. Unplanned pregnancy women were 0.5 times at risk to have a high risk of prenatal depression [AOR=0.50, 95% CI (0.50 (0.26-0.96)] at 8-12 weeks of pregnancy, and 0.4 times at 24 – 28 weeks of pregnancy [AOR=0.40; 95% CI (0.21-0.81)] linked to women with planned pregnancy. History of depression were 5.4 times [AOR=5.42, 95% CI (2.09-14.08)] at 8-12 weeks of pregnancy and 7.2 times at 24 – 28 weeks of pregnancy [AOR=7.19; 95% CI (2.50-20.70)] possibly to have prenatal depression than women without a history of depression.

Table 3-3 Logistic regression analysis of risk factors for depression symptoms among pregnant Omani women at week 8-12 and week 24 -28 of pregnancy in the study Odds Ratio (95% CI):

	at weeks 8-12 of pregnancy		**at weeks 24-28 of pregnancy**	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***P***
Degree or higher education level	6.55(1.70-25.13)	**0.01**	8.13(1.84-35.82)	**0.01**
Is the pregnancy planned? (No)	0.50(0.26-0.96)	**0.04**	0.40(0.20-0.81)	**0.01**
History of depression (yes)	5.42(2.09-14.08)	**0.00**	7.19(2.50-20.70)	**0.00**
Variable(s) entered in Step 1: Age categories, educational level, occupational status, annual household income, housing tenure, duration of marriage, and whether the pregnancy was planned. History of depression, family history of depression, physical activity, parity category, previous pregnancy complications, mode of delivery in previous pregnancies, BMI in registration category.				

3.4.3 Prevalence of anxiety symptoms

Table 3-4 shows the prevalence of anxiety symptoms and the associations between such symptoms during pregnancy and sociodemographic variables among sample of pregnant Omani women. Using a cut-off point of 6 in (EPDS-3A), the proportion of women who showed anxiety symptoms was 24.8% between 8 and 12 weeks of pregnancy and 26.1% between 24 and 28 weeks.

In terms of univariate analysis, anxiety symptoms in pregnant women between 8 and 12 weeks, in the case of those with a history of depression ($p = 0.0001$) and in cases of unplanned pregnancy ($p = 0.043$), were significantly linked to anxiety symptoms in the study sample. The current study found that, compared to other studies, low physical activity in pregnancy and a history of depression are linked to high anxiety symptoms at 24-28 weeks. None of the following variables: maternal age, occupational status, maternal education, monthly family income, duration of marriage, family history of depression, parity, obstetric complications in previous pregnancies, mode of delivery in previous pregnancies, BMI at registration, which were involved in the bivariate analysis, were significantly linked with maternal anxiety symptoms among the study sample.

Table 3-4 Associations between antenatal anxiety and sociodemographic gestational characteristics variables among pregnant Omani women:

Variables	8 – 12 weeks of pregnancy N = 302						24 – 28 weeks of pregnancy N = 272					
	Non-anxiety (EPDS-3A ≤ 5) n=227(75.2%)		Anxiety (EPDS-3A ≥ 6) n=75 (24.8%)				Non-anxiety (EPDS-3A ≤ 5) n=75 (73.9%)		Anxiety (EPDS-3A 6) n=71 (26.1%)			
	N	%	n	%	(*df*) χ^2	*p*	n	%	n	%	(*df*) χ^2	*P*
Age (Y)					1.062	0.781					2.409	0.492
< 20	8	3.5	1	1.3			5	2.5	3	4.2		
20 – 30	100	44.1	35	46.7			89	44.3	32	45.1		
30 – 40	105	46.3	35	46.7			92	45.8	34	47.9		
> 40	14	6.2	4	5.3			15	7.5	2	2.8		
Educational level					1.831	0.400					5.062	0.080
Basic Education	20	8.8	8	10.7			21	10.4	4	5.6		
Secondary Education	105	46.3	28	37.3			94	46.8	26	36.6		
Degree or higher	102	44.9	39	52			86	42.8	41	57.7		
Occupational Status					0.030	0.862					0.523	0.469
Employed	69	30.4	22	29.3			63	31.3	19	26.8		
Unemployed	158	69.6	53	70.7			138	68.7	52	73.2		
Retired												
Annual household income					1.683	0.431					2.937	0.230
Less than 500 OR	98	43.2	36	48			90	44.8	31	43.7		
500 OR – 1000 OR	91	40.1	31	41.3			76	37.8	33	46.5		
Above 1000	38	16.7	8	10.7			35	17.4	7	9.9		
Housing tenure					3.181	0.204					4.286	0.117
Rent house	58	25.6	20	26.7			47	23.4	23	32.4		
Homeowner	94	41.4	23	30.7			83	41.3	20	28.2		
Live with the husband or wife family	75	33	32	42.7			71	35.3	28	39.4		
Duration of Marriage					4.111	0.253					3.855	0.278

Less than 2 years	45	19.8	12	16			36	17.9	16	22.5		
Less than 5 years	49	21.6	12	16			42	20.9	17	23.9		
Less than 10 years	74	32.6	34	45.3			69	34.3	27	28		
older than 10 years	59	26	17	22.7			54	26.9	11	15.5		
Planned pregnancy					4.112	***0.043***					0.511	0.475
Yes	159	70	43	57.3			134	66.7	44	62		
No	68	30	32	42.7			67	33.3	27	48		
Physical Activity					2.537	0.469					7.601	***0.05***
No	114	50.2	44	58.7			99	49.3	44	62		
30 minutes for at least 2/ week	69	30.4	17	22.7			63	31.3	13	18.3		
30 minutes for at least 3/week	20	8.8	8	10.7			15	7.5	9	12.7		
30 minutes for at least 5/week	24	10.6	6	8			24	11.9	5	7		
History of Depression					15.346	***0.001***					7.392	***0.001***
Yes	15	6.6	17	22.7			16	8	14	19.7		
No	212	93.4	58	77.3			185	92	57	80.3		
Family history of depression					0.066	0.797					0.017	0.897
Yes	19	8.4	7	9.3			18	9	6	8.5		
No	208	91.6	68	90.7			183	91	65	91.5		
Parity					0.715	0.700					0.328	0.849
1	54	23.8	15	20			45	22.4	18	25.4		
2	55	24.2	17	22.7			47	23.4	17	23.9		
3+	118	52	43	57.3			109	54.2	36	50.7		
Obesity BMI					1.180	0.758					1.029	0.794
Underweight	18	7.9	5	6.7			15	7.5	6	8.5		
Normal	53	23.3	22	29.3			50	24.9	17	23.9		
Overweight	65	28.6	21	28			62	30.8	18	25.4		
Obesity	91	40.1	27	36			74	36.8	30	42.3		

Obstetric complication Previous Pregnancy					4.084	0.253					1.100	0.777
Preeclampsia (PE)	8	3.5	0	0			7	3.5	1	1.4		
Miscarriage	53	23.3	23	30.7			47	23.4	17	23.9		
Preterm	12	5.3	3	4			8	4	4	5.6		
Mode of Delivery in previous pregnancies					1.069	0.586					0.365	0.833
Normal Delivery (SVD)	121	53.3	43	57.3			109	54.2	36	50.7		
Caesarean section (CS)	64	28.2	22	29.3			58	28.9	21	29.6		
No previous pregnancies	42	18.5	10	13.3			34	16.9	14	19.7		

Table 3-5 shows the logistic regression results for predictors of anxiety symptoms during pregnancy using the odds ratio (95% CI) between the study samples. Multivariate logistic regression analysis for anxiety symptoms showed that the level of education of pregnant women was significantly linked with a higher rate of anxiety symptoms, as the odds of these symptoms were 3.5 times higher among pregnant women who had completed university education [AOR=3.4; 95% CI (0.91-13.17)] at 24 - 28 weeks of pregnancy than to mothers who had received secondary education. Women with unplanned pregnancies were 0.4 times at risk to have a high rate of anxiety symptoms during pregnancy between 8 and 12 weeks [AOR=0.46; 95% CI (0.24-0.89)] than those who had planned their current pregnancy. Women with a history of depression were 6 times [AOR=5.92, 95% CI (2.27-15.78)] at 8-12 weeks of pregnancy and 4.1 times at 24 – 28 weeks of pregnancy [AOR=4.13; 95% CI (1.56-10.93)] more likely to have a higher anxiety symptom during pregnancy than to women without a history of depression. Pregnant women with low physical activity during pregnancy (30 minutes for at least two times/week) were 0.4 times [AOR=0.45, 95% CI (0.21-0.96)] at risk to have a high rate of anxiety symptoms between 24 and 28 weeks of pregnancy compared to women with increased physical activity during pregnancy.

Table 3-5 Logistic regression analysis of risk factors for anxiety symptoms among pregnant Omani women at week 8-12 and week 24 -28 of pregnancy in the study Odds Ratio (95% CI):

	at weeks 8-12 of pregnancy		at weeks 24-28 of pregnancy	
	OR (95% C.I.)	***P***	**OR (95% C.I.)**	***P***
Degree or higher education level	1.69(0.54-5.27)	0.36	3.46(0.91-13.17)	**0.05**
Whether the pregnancy was planned? (No)	0.46(0.24-0.88)	**0.02**	0.71(0.36-1.40)	0.32
History of depression (Yes)	5.98(2.27-15.78)	**0.00**	4.13(1.56-10.93)	**0.00**
physical activity for 30 minutes for at least 2/ week	0.74(0.37-1.51)	0.41	0.44(0.20-0.96)	**0.04**
Variable(s) entered in Step 1: Age categories, educational level, occupational status, annual household income, housing tenure, duration of marriage, and whether the pregnancy was planned. History of depression, family history of depression, physical activity, parity category, previous pregnancy complications, mode of delivery in previous pregnancies, BMI in registration category.				

3.4.4 Pregnancy outcomes

Table 3-6 shows the study's pregnancy and newborn outcomes of sample of pregnant Omani women (n = 242). In the current pregnancy, among all participants in the study, 19% had GDM, and 11.6% had preterm delivery. The mean gestation age in days at delivery was 270.8 days. Around 13% of the pregnant women had a LBW delivery (LBW <2500 g), and 7% had delivered small babies for their gestational age. The mean length of the infant (cm), head circumference (cm) and birth weight (gm) at delivery were 49.8 cm, 33.4 cm and 2984 gm, respectively. Finally, the mean range of the Apgar score at 1 and 5 minutes was 8.8 and 9.9, respectively.

Table 3-6 Pregnancy and neonatal outcomes of the Omani pregnant women in the study:

Variables	N (242)	%
GDM	59	19.2
Normal Delivery (SVD)	171	70.5
Caesarean section (CS)	71	29.5
Full term delivery	214	88.4
Preterm delivery	28	11.6
BMI at delivery		
Underweight	23	7.6
Normal	75	24.8
Overweight	86	28.5
Obesity	118	39.1
Low birth weight		
Low weight LBW <2500 g	30	12.4
Small Gestational Age	17	7
Average Gestational Age	208	86
Large Gestational Age	17	7
Gestational weight gain category		
Adequate	105	43.4
Inadequate	120	49.6
Excessive	17	7
	Mean	**SD**
Gestation age by days at delivery	270.85	12.1
Body Mass Index (BMI) at delivery	31.5	6.76
Infant length (cm)	49.85	3.177
Birth weight (gm)	2984.31	527.7
Head circumference (cm)	33.41	1.80
Apgar score at 1 minute	8.8	0.77
Apgar score at 5 mins	9.9	0.46

Table 3-7 and Table 3-8 show the association between depressive and anxiety symptoms at 8-12 weeks and 24-28 weeks of pregnancy with pregnancy outcomes. At 24 to 28 weeks of pregnancy, an increased incidence of depressive symptoms was reported in pregnant women with GDM (22.8% vs 12%; P <0.04) compared to women without GDM. Anxiety and depressive symptoms were not significantly linked with spontaneous preterm or PE at 8-12 weeks or 24-28 weeks of pregnancy.

Table 3-7 Associations between depression symptoms at 8 – 12 weeks and 24 – 28 weeks of pregnancy with pregnancy outcome:

	at week 8-12 of pregnancy					at week 24-28 of pregnancy				
Variable	**Non-depressive** (EPDS ≤12) (n=212) (70.2%)		**Depressive** (EPDS)≥13 (n=90) (29.8%)			**Non-depressive** (EPDS ≤12) (n=189) (72.5%)		**Depressive** (EPDS) ≥ 13 (n=83) (27.5%)		
	N	%	N	%	*p*	N	%	N	%	*p*
Spontaneous preterm					0.9					0.63
Yes	11	5.2	5	5.6		12	6.3	4	4.8	
No	201	94.8	85	94.4		177	93.7	79	95.2	
GDM					0.57					**0.04**
Yes	45	21.2	14	15.6		10	12	43	22.8	
No	167	78.8	76	84.4		146	77.2	73	88	
PE					0.25					0.55
Yes	11	5.2	2	2.2		10	5.3	3	3.6	
No	201	94.8	88	97.8		179	94.7	80	96.4	

Table 3-9 shows the logistic regression analysis aiming to assess the associations linking depression and anxiety symptoms at weeks 8 – 12 and 24 – 28 weeks of pregnancy with GDM, PE, and spontaneous preterm birth (N = 242). Similarly, to the analysis in Table 8, women with depression symptoms at 24-28 weeks of pregnancy had a 2.15-fold higher chance of developing GDM (GDM) during pregnancy (95% CI: 1.02 – 4.52, $p = 0.04$) than women without depression symptoms during pregnancy. There was no association between PE or spontaneous preterm and maternal depression and anxiety symptoms. These results were replicated after adjusting for potential confounders.

Table 3-8 Associations between anxiety symptoms at 8 – 12 weeks and 24 – 28 weeks of pregnancy with pregnancy outcome:

	at week 8-12 of pregnancy					at week 24-28 of pregnancy				
Variable	**Non-anxiety** (EPDS ≤12) (n=212) (70.2%)		**Anxiety** (EPDS)≥13 (n=90)(29.8%)			**Non-anxiety** (EPDS ≤12) (n=189) (72.5%)		**Anxiety** (EPDS) ≥ 13 (n=83) (27.5%)		
	n	%	n	%	*p*	n	%	N	%	*p*
Spontaneous preterm					0.56					0.91
Yes	13	5.7	3	4		12	6	4	5.6	
No	214	94.3	72	96		189	94	67	94.4	
GDM					0.58					0.09
Yes	46	20.3	13	17.3		44	21.9	9	12.7	
No	181	79.7	62	82.7		157	78.1	62	87.3	
PE					0.42					0.37
Yes	11	4.8	2	2.7		11	5.5	2	2.8	
No	216	95.2	73	97.3		190	94.5	69	97.2	

Table 3-9 Associations between depression and anxiety symptoms with GDM, preterm and PE (N = 242):

	GDM			S Preterm			PE		
	N=59	OR (95%CI)	***p***	**N=16**	OR (95%CI)	**N=*p***	**13**	OR (95%CI)	***p***
Depression at 8 – 12 weeks of Pregnancy									
No	45	-		21	-		10	-	
Yes	14	1.46(.76-2.83)	0.25	7	0.93 (.31-2.8)	0.89	3	2.4 (.52-11.09)	0.25
Depression at 24– 28 weeks of Pregnancy									
No	43	-		22	-		9	-	
Yes	10	2.15 (1.02-4.52)	**0.04**	6	1.33 (.41-4.28)	0.62	4	1.49 (.39-5.5)	0.55
Anxiety at 8 – 12 weeks of Pregnancy									
No	46	-		24	-		10	-	
Yes	13	1.21(.61-2.39)	0.57	4	1.4 (.4 -5.2)	0.49	3	1.85 (.4-8.58)	0.42
Anxiety at 24– 28 weeks of Pregnancy									
No	44	-		22	-		9	-	
Yes	9	1.93(.88-4.19)	0.09	6	1.06 (.33-3.41)	0.11	4	1.99 (.43-9.2)	0.37

Table 3-10 shows the associations between depression and anxiety symptoms at weeks 8-12 and 24-18 weeks of pregnancy, with gestational duration in weeks and the birthweight centile in the linear regression analyses.

The regression models (Table 3-10) show that the appearance of antenatal depression and anxiety symptoms is correlated with a higher probability of having a lower gestational age at delivery. For example, lower gestational age was associated with depression symptoms at 24 – 28 weeks of pregnancy (p = 0.001) (β = 0.68; 95% CI 0.013 to 1.290) and anxiety symptoms at 8 – 12 weeks of pregnancy (p = 0.02) (β = 0.75; 95% CI 0.106 to 1.3) and at 24 – 28 weeks of pregnancy (p=0.002) (β = 0.98 95% CI 0.343 to 1.616).

Table 3-10 Liner regression investigated the association between depressive and anxiety symptoms with gestational duration (days) and customised birthweight centile:

	Gestational age by (weeks)		**Customised birthweight centile**	
	β (95%CI)	***p***	**β (95%CI)**	***p***
Depression at 8 – 12 weeks of pregnancy				
No	-		-	
Yes	0.475(-0.136-1.086)	0.196	0.008 (-7.343-8.267)	0.907
Depression at 24– 28 weeks of pregnancy				
No	-		-	
Yes	0.676 (0.013-1.290)	**0.003**	0.8 (-6.922-8.845)	0.81
Anxiety at 8 – 12 weeks of pregnancy				
No	-		-	
Yes	0.751(0.106-1.39)	**0.023**	0.914 (-7.8-8.706)	0.914
Anxiety at 24– 28 weeks of pregnancy				
No	-		-	
Yes	0.98 (0.343-1.616)	**0.003**	0.042 (-5.548 -11.025)	0.516

3.5 Discussion

3.5.1 Prevalence of Depression and Anxiety Symptoms

The current study aims to evaluate the incidence and risk factors linked with prenatal depression and anxiety symptoms among sample of (N=300) Omani women in the first and second trimesters of pregnancy. Therefore, it can significantly influence the identification of depressive episodes during pregnancy among Omani women. The current study found that around 30% of the study sample was identified as having antenatal depression based on cutoff-off 13 on the EPDS scale.

The prevalence of depressive symptoms in this cohort of sample of pregnant Omani women was higher than findings from other estimations of depression symptoms among Omani women - 12% (55) and 24.3% (56). In the Arabic world, maternal depression is becoming a more recognised condition, with prevalence rates ranging from 10% - 37% (45–49). In Gulf countries, where women have a culture similar to that of Omani women, the highest prevalence rate, 37%, was recorded in Bahrain (50). In a cross-sectional study to evaluate the incidence of prenatal depression among women in Saudi Arabia, 31.9% of the study sample were shown to have high depression symptoms during pregnancy (639). The United Arab Emirates has rates between 10% and 20% (51,52); in Kuwait, the rate is 11.7% (53); and in Qatar, the rate is between 17.6% and 21% (54,640).

Furthermore, the findings of the study also indicate that the prevalence of antenatal depression in a sample of Omani women (N=300) is higher than the incidence reported by other countries such as the United States (16.6%), Australia (16.9%), Brazil (13.5%), and Turkey (21.6%) (640–644), but almost similar to the prevalence assessed in Asia

and Africa in countries such as China (28.5%), India (35.7%), North Tanzania (33.8%) and Northwest Ethiopia (31.2%) (645–648).

In our study, the prevalence of anxiety in a sample of Omani women (N=300) was 24.8% and 26.1% at the two assessment points using a cutoff of 6 in (EPDS-3A) (595). These results were similar to figures from a meta-analysis of anxiety symptoms among pregnant women that reported a prevalence of 25% (649) and higher than other estimations of prenatal anxiety in Europe, 15% and 18% (650,651). However, the incidence of maternal anxiety among women in our study was lower than the results of studies aimed at evaluating depression and anxiety symptoms among women during the Covid-19 pandemic, for example, 34% in Egypt (652), 29% in China (653), and 57% in Canada (654).

Methodological and study design differences, including the mental health assessment scale and economic and sociodemographic differences, could be related to the variance in depression and anxiety symptoms between studies in different countries. Other risk factors and cultural differences can influence anxiety and depression symptoms during pregnancy. The relatively significant increase in maternal depression in our study could be attributed to the investigation carried out during the Covid-19 pandemic, mainly during the lockdown time in Oman. Several researches and literature in various regions of the world have reported that the most significant effect of the COVID-19 pandemic was poor mental health outcomes, especially the high rate of anxiety and symptoms of depression among pregnant women (652–655).

In this study, the assessment of prenatal anxiety and depression symptoms among Omani women was measured at two different assessment points, 8-12 weeks and 24-28 weeks

of pregnancy. Depression and anxiety symptoms increased slightly during pregnancy. Overall, the prevalent rate of depression and anxiety symptoms at 8-12 weeks was 29.8% and 24.8%, respectively, rising throughout pregnancy to 30.5% and 26.1%, respectively, at 24 – 28 weeks of pregnancy using 13 on the EPDS scale for depression and 6 on EPDS-3A. Consistent with the results of this study, the use of EPDS at two assessment points during pregnancy has shown that depression and anxiety symptoms increase over time (649,656–658).

The American College of Obstetricians and Gynecologists recommends including the screening for prenatal depression in the healthcare system of obstetricians and gynecologists in developed countries (659). Given the reasonably high incidence of anxiety and depressive symptoms among pregnant Omanis in the present study, the Oman Ministry of Health should plan to apply routine screening for prenatal depression and anxiety symptoms as a form of routine maternal care services. The early diagnosis of women with depression and anxiety symptoms would allow healthcare professionals to offer support to these women and potentially decrease the rate and impact of mental health disorders and their related complications in Oman (48,584).

3.5.2 Predictors of Depression and Anxiety Symptoms

The current cohort of Omani women indicated that the type of accommodation, history of depression, unplanned pregnancy, and level of education are significantly associated with antenatal depression. The multivariate analysis confirmed that unplanned pregnancy, physical activity by pregnant women, history of depression, and level of education are the strongest predictors of high anxiety symptoms among the study sample.

Pregnant women who live in the home of their parents or that of their husband's family may be exposed to psychological pressures due to a conflict with a family member, which could increase the risk of antenatal depression. For example, the mother-in-law often interferes with pregnancy care, and the mother may not get her husband's full support when she complains about his mother's behaviour (660). Therefore, the absence of partner support could be a significant risk factor for prenatal depression (661). In addition to child care, a family member's illness markedly increases the mother's responsibilities regarding her family, which can increase stress (55). These factors lead to lower marital satisfaction. Previous studies showed that new mothers with lower marital satisfaction had a high risk of suffering from antenatal depression (662–665).

Studies have found that a history of depression is a critical predictor of experiencing depressive and anxiety symptoms among sample of pregnant Omani women and is consistent with our findings (38). However, this could be a severe problem in addressing antenatal depression in Oman. Women in Oman, like many other Arab women, have a low rate of reporting a history of depression because they believe that mental health disorders are a social stigma (666). They tend to deny the presence of any depressive symptoms and refuse to seek medical attention, as they feel ashamed of being diagnosed with a psychological disorder. They trust their faith or consult religious leaders for assistance (529). Antenatal depression and anxiety symptoms appeared to be associated with low levels of education (667,668). Interestingly, in the current cohort study, sample of pregnant Omani women who had completed a university education or higher had a higher rate of anxiety and depression symptoms than women with lower levels of education. This result was consistent with those of other studies (669–671).

Previous studies have provided evidence that highly educated women appear to have little concern, cry, suffer from self-harm, and do not see the funny side of things (670). However, until now, there has been no explanation for the association of antenatal depression with a higher educational level. However, such women could have a great deal of health knowledge and be more anxious about psychological health.

Unplanned pregnancy was shown to predict depressive and anxiety symptoms among the current cohort of women. Unplanned pregnancies are associated with depression and anxiety symptoms due to challenges in balancing maternal needs and personal or work tasks (672). Pregnant women who do not plan for pregnancy do not initiate maternal care and may have an unbalanced psychosocial environment, including their relationship with their partner (673). On the other hand, women with planned pregnancies are more likely to be prepared for pregnancy and the demands of childbirth (56).

3.5.3 Pregnancy outcomes

Antenatal depression increases the odds of poor pregnancy outcomes, including preterm birth, LBW, GDM, PE, and the need for CS (674). Regarding GDM, the study results also showed that antenatal depression at 24-28 weeks of pregnancy is significantly related to GDM. This finding is supported by recent meta-analyses and reviews that indicate the association between depression during pregnancy and a high rate of GDM among pregnant women (675–677). GDM carries a risk of poor mental health and cognitive and learning disabilities in the newborn; moreover, depression symptoms are present in 1/4 of people with T2DM (678). The causal association between depression and GDM remains unclear. However, several pieces of research support an inflammatory process in women with antenatal depression and GDM (679). For example, inflammation-related

cytokines are associated with depression and type 2 diabetes, affecting pancreatic B cells and leading to insulin resistance (680). Furthermore, activation of the hypothalamic-pituitary-adrenal axis by cytokines effectively regulates the body's response to stress. Additionally, depression can lead to a sedentary lifestyle and a poor diet, increasing the risk of T2DM (681).

The incidence of maternal anxiety and depression was associated with a reduction in gestational age by one week at the time of delivery. The highest probability of earlier delivery was observed in anxiety symptoms at 24-28 weeks of pregnancy, almost double compared to the control group. However, our finding that there is no association between perinatal anxiety and depression with PE and preterm birth is inconsistent with previous research examining these associations. Several studies have revealed that women with high prenatal anxiety or depression symptoms are more likely to have PE and experience preterm birth than women without such disorders (682–684). This could be explained by the small number in the PE group (n=13) and the preterm group (n=13). More research is warranted with many pregnant Omani women to better evaluate the causality of the association.

The study result shows that there was no relationship between depression and anxiety symptoms with the delivery of an SGA infant. Limited evidence supports a correlation between antenatal depression and the increased probability of giving birth to SGA, particularly during mid-pregnancy (685–687).

To our knowledge, customised centiles have not been used to report measures of SGA among women with symptoms of maternal anxiety and depression. SGA rates were

notably higher when calculated using customised centiles compared to population centiles (688). Infants with SGA who were previously unrecognised as AGA due to their classification as AGA according to population centiles had similar morbidity rates to infants identified as SGA through both methods. It is essential to approach these findings with caution, as the study only involved a small number of babies with SGA. However, the data are consistent with similar research in other populations, which found high morbidity rates among customised infants with SGA (688–690).To confirm these findings, further studies with a well-powered design are warranted.

3.5.4 Strengths and Limitations

This study's strengths lie in its ability to gather reports of antenatal depression and anxiety symptoms during early pregnancy, ensuring that the report was not influenced by pregnancy outcomes. However, there are a few possible limitations to this research study. First, the study relied on self-reported questionnaires for data. This could have resulted in recall bias and unreliable estimates of the prevalence of antenatal depression. Second, the study lacked definite diagnostic standards for antenatal depression. The EPDS is primarily a screening tool and is not intended for diagnosis. Future studies should use periodic diagnostic tests to accurately detect psychiatric disorders in expectant mothers. Third, all participants in the study will have a similar possibility of being misclassified with respect to their status of results and their exposure status. This could lead to bias in terms of the link between outcome and exposure. Fourth, despite our efforts to account for potential confounding variables, there is still a chance that unmeasured factors could have impacted our findings, leading to residual confounding. Further research is necessary to establish any potential causal link between depressive symptoms and other

variables. Lastly, we decided to use a convenient non-probability sampling procedure in a sample of pregnant women with enough time and willingness to answer all interview questions. Non randomised sampling restricts generalising about the population, as some of its features may not be well represented in the sample. Pregnant women who experience multiple responsibilities and face elevated stress levels may have hesitated to participate in the research.

3.6 Conclusions

This is the first study to attempt to assess the incidence and predictors linked with depression and anxiety symptoms among sample of pregnant Omani women. The findings indicated that maternal depression and anxiety symptoms were higher than other estimates carried out in Oman. The level of education, history of depression, the type of accommodation, and the unplanned pregnancy were significantly linked with antenatal depression and physical activity, a predictor of high anxiety and depression symptoms among the study sample. The study results and those of other studies (55,56) support the need for increased public health initiatives to screen and treat depression and anxiety symptoms in sample of pregnant Omani women as a means of prevention of adverse pregnancy outcomes. Further extensive research is required to report the incidence of prenatal anxiety and depression in Oman and to better assess the causality of the association.

Chapter 4: Maternal FAs intake and levels and the presence of depressive and anxiety symptoms

4.1 Introduction

Mental health disorders such as depression have adverse social and economic consequences for individuals and society. Depression has been shown to decrease the quality of life and intensify disability (5). Pregnant and lactating women are particularly vulnerable to increased episodes of depression (17). Observational and experimental research has hypothesised that nutrition could play an essential part in the progress of mental disorders, including depression (691).

The brain is a fat rich organ with a high dependence on omega-3 FAs, the status of which is dependent on the diet (121). Studies indicate the outcome of the health benefits of omega-3 FAs in metabolic conditions, Cardiovascular disease (CVD), and cancer (692–697). Recently, research has explored the positive effects of omega-3 FAs on mental disorders, including depression (534). According to the ALSPAC study, which examined over 14,000 pregnancies, the amount of omega-3-rich fish and sea foods consumed by the mother during pregnancy had a direct correlation with the verbal IQ, social, and behavioural scores of children at 8 years of age (177). Drawing from existing evidence (698,699), the beneficial outcome of using omega-3 FAs in the management of psychiatric illnesses has been highlighted, suggesting the potential to find a rational approach to treatment.

Recent evidence has shown a relationship between omega-3 FAs bioavailability in neurological disorders, including depression (237). The lack of omega-3 FAs is

associated with various pathological conditions, including antioxidant system deficiency (203). Low omega-3 FAs were also reported in red blood cell membranes in depressed patients (700,701). This has been hypothesised to be due to inadequate omega-3 FAs or a yet-to-be-charted genetic abnormality (567).

This lack has resulted in several unfavourable health impacts on mothers and children, including altered obstetric risk and fetal growth restrictions, such as poor neurodevelopment and cognitive development (25). LC-PUFAs, ArA and DHA, are crucial for proper brain and nervous system development in the fetus (703). Low levels of DHA in pregnant women can lead to maternal depression (254,259). Therefore, adequate DHA intake during pregnancy is crucial for the fetus and healthy neural growth of the newborn (117).

Indeed, several researchers have indicated that pregnant and nursing mothers with low omega-3 FAs are at significant risk of prenatal and postnatal depression and have recommended higher intakes (26,117,704). Furthermore, few observational studies have correlated information on omega-3 FAs, especially DHA, with increased rates of depressive symptoms during pregnancy (262). Others have assessed this relationship by measuring erythrocyte membrane, serum or plasma omega-3 FAs levels, and related them to depressive symptoms in pregnant women, reporting that low DHA concentrations or higher omega-6/omega-3 FAs ratios are consistent with a high rate of antenatal depression (263,267).

Current observational studies and RCTs have assessed the potential benefits of omega-3 FAs consumption on maternal depression (260). However, the exact relationship has not yet been concluded (117).

4.2 Aim

The objective was to evaluate FAs in maternal and cord erythrocytes as a proportion of total FAs and in absolute quantities.

The general objectives of this chapter are to examine the association between (i) omega-3 FAs intake and the omega-3 FAs of the red blood cell membrane of the maternal (during pregnancy) and fetal (cord) red blood cell membranes. (ii) Maternal FAs intake and levels and depressive and anxiety symptoms in Omani pregnant women.

4.3 Methods

This prospective cohort study recruited 300 pregnant Omani women receiving maternal care at Al Buraimi Hospital, Oman. Oman is the second largest country in the Arabian Gulf on the Arabian Peninsula. The Ethics and Research Committee Board of the Oman Ministry of Health approved the study protocol on 1 July 2019 (MoH/CSR/19/9668) (The ethical approval is attached in Appendix 4). Participants were recruited from the antenatal clinics of Al Buraimi Hospital and the Primary Health Center in Al Buraimi, which serves the local community of the Al-Buraimi Governorate .In summary, patient electronic medical records were reviewed to obtain antenatal, intrapartum, and postpartum data. Maternal characteristics and complications were evaluated using general health questionnaires. Pregnancy and neonatal outcome records were collected from mothers and their offspring and used to achieve the aims and objectives of the study. The methods used, including inclusion and exclusion criteria, were described in detail in Chapter 2.

After getting fully informed written consent from pregnant women, the level of depression and anxiety was assessed at 8-12 and 24-28 weeks of pregnancy using a modified self-

administered Arabic version of the Edinburgh Postnatal Depression Scale (EPDS) translated by (48). The estimation dietary intake of the participants in terms of EPA and DHA was measured using an altered version of the abbreviated FFQ of 7 questions designed by (536) to determine daily intake in healthy adults. The author obtained permission to use and translate the abbreviated FFQ (Appendix 1).

Fasting blood samples (5ml) were collected in a preservation tube containing ethylene diamine tetraacetic acid (BD Vacutainer® EDTA tubes) at 8-12 weeks of gestation. The erythrocyte membrane and plasma FAs were isolated and stored in the -80°C freezer until needed for analysis. In this research project, lipid extraction was done from maternal and cord erythrocyte samples following the Folch et al. approach, as shown in the Materials and Methods section in Chapter 2. After the FAs were extracted, the FAMEs were separated using GC. The separated compounds were then loaded into an autosampler ampule and repeatedly injected into the Agilent GC-7890A device equipped with an MS-Premium low bleed column (60 m x 0.32 mm ID, 0.25 µm film, BP20) (SGE Analytical Science, Australia) range with a maximum temperature of 260°C. The carrier gas used was hydrogen, and FAMEs were detected by evaluating their retention times with a recognised standard, the 37-component FAMEs mix (Sigma Aldrich). A computer-based data chromatography system was used to determine the peak areas in the sample in terms of percentage values (Agilent Open LAB chromatography data system, Scientific Software Inc., San Ramon, CA). The peak areas were calculated based on the percentage of total peaks identified.

4.3.1 Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 21, developed by IBM Corp., Chicago, Illinois, USA. Descriptive statistics were used to describe the socioeconomic, demographic, and psychological data of pregnant women. For continuous variables, the results were expressed as means, and standard deviations (SD), while categorical variables were presented as frequencies and percentages. Histograms and box plots were produced with respect to continuous variables to study the distribution and identify outliers. To investigate the link between antenatal depression and anxiety symptoms with sociodemographic factors, we conducted a univariate analysis using Pearson's chi-square test. A p-value of ≤0.05 was deemed statistically significant.

Logistic regression analysis tests were set to evaluate 95% confidence intervals (CI) and odds ratios (OR). Both tests were used to explore the relationship with anxiety symptoms (EPDS-3A score <6 v. EPDS-3A score ≥6), depressive symptoms (EPDS score <13 v. EPDS score ≥13), and pregnancy outcomes.

To adjust for potentially confounding influences, additional analysis was performed using a sequence of multiple regression statistics tests for variables that showed substantial links with depression and anxiety symptoms at the $p \leq 0.05$ level. The enter method, followed by a removed regression method was used in the analysis. All variables are entered and then we try to eliminate the variables with less contribution to the total variance. Potential confounder variables included maternal age, marital status, parity, annual household income, history of depression, anxiety disorder, mode of previous

deliveries, employment status, educational level, physical activity, and BMI prior to pregnancy.

4.4 Results

The sociodemographic and medical characteristics of the study participants, including the prevalence of anxiety and depressive symptoms and data on pregnancy and newborn outcomes, were described in Chapter 3.

4.4.1 Fish and Omega 3 FAs intake

Table 4-1 shows that 39.4% of the women in the study reported the fish intake in 1-2 portions per week. at 8 -12 weeks of pregnancy, while 42.1% of the 302 pregnant women reported consuming more than two portions per week. Only 18.5% of the study sample reported that they did not eat fish at all. At 24 to 28 weeks of pregnancy, 39.3% of the participants reported having eaten 1-2 portions per week was reported by 39.3% of the participants, while 41.2% of the 302 pregnant women reported having eaten more than two portions per week. Only 19.5% of the study sample reported that they did not eat fish at all. The estimation daily median (interquartile range) nutritional intake of omega-3, FAs DHA and EPA at 8-12 and 24-28 weeks of gestation is shown in Table 4-1. The daily maternal median intake of DHA and EPA at 8 – 12 weeks of pregnancy is estimated to be approximately 165 mg/d and 116.5 mg/d, respectively. The median daily intake of the omega-3 index is 275.5 (mg/day). However, the daily maternal median intake of EPA and DHA at 24 to 28 weeks of pregnancy is estimated to be approximately 161 mg/d and 112 mg/d, respectively. The median total daily intake of the omega-3 index is 273.5 (mg/day). Only 20 pregnant women (6.6%) reported taking dietary supplements containing omega-3 FAs during pregnancy. Table 4-2 shows the relationship between fish consumption per

week at 8-12 weeks of pregnancy and the daily median nutritional intake of omega-3 FAs. There were significant differences in the mean intake of EPA and DHA in the fish intake category (p=0.01). As expected, the median intake of DHA, EPA and the omega-3 index were higher in pregnant women with the highest fish intake per week. Furthermore, pregnant women who ate no or 1-2 fish per week were expected to have a lower daily median intake of DHA, EPA and total omega-3 index FAs.

Table 4-1 Distribution of daily intake of fish and omega 3 FAs and dietary supplements:

	at weeks 8-12 of pregnancy		**at weeks 24-28 of pregnancy**	
Fish intake during pregnancy	N = 302	%	N = 272	%
Non/week	56	18.5	53	19.5
1-2/week	119	39.4	107	39.3
≥ 3/week	127	42.1	112	41.2
Omega-3 FAs	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**
Total DHA (mg/d)	165(76-252)	181.2(124.2)	161(74-251)	177.4(122)
Total EPA (mg/d)	116.5(66-175)	133.5(94.9)	112(66-172)	130.4(88.9)
Omega-3 EPA + DHA (mg/d)	275.5 (149.5-427)	314.7 (214.9)	273.5 (138-424)	307.8 (207.1)
Dietary supplements omega-3 FAs	20	6.6%	17	6.3%
Dietary supplements vitamins	125	41.4%	111	40.8%

Table 4-2 Relationship between fish intake (fish portion/week) and the estimated amount of the median daily intake of EPA and DHA:

Daily intake of Omega-3 fatty acids	**Fish Consumed (portion/wk)**						
	None/week		**1-2/week**		**≥ 3/week**		
	Median	**Mean**	**Median**	**Mean**	**Median**	**Mean**	***P***
Total DHA (mg)	33	46.6	129	131.2	259	287.4	<.001
Total EPA (mg)	43	50.8	97	97.3	177	203.8	<.001
Total n-3 EPA + DHA (mg/day)	75	97.4	224	228.5	440	491.2	<.001

Table 4-3 shows the relationship between confounding factors and fish intake by the number of portions per week and the median total daily nutritional intake of DHA and EPA FAs among women in the study. Fish intake per week was inversely related to a history of depression ($p = 0.04$). Furthermore, the median intake of EPA, DHA and the omega-3 index were inversely related to the history of depression ($p = 0.02$, $p=0.04$ and $p=0.02$) respectively. The median EPA dietary intake was positively associated with the accommodation type, specifically in the group of women who live in their own home (homeowners) - $p = 0.02$ and $p = 0.04$, respectively. Then again, DHA and EPA were associated or showed a trend with physical activity among the study sample as shown here ($p = 0.04$, $p = 0.05$ and $p = 0.05$) respectively. None of the other variables of the sociodemographic and clinical characteristics variables (educational level, family income, maternal age, occupational status, duration of marriage, planned pregnancy, family history of depression, obstetric complications, previous pregnancy, mode of delivery in previous pregnancies, and BMI at registration) were significantly associated with fish and omega-3 FAs intake.

Table 4-3 The association between sociodemographic and gestational characteristics of Omani pregnant women in the study (N=302) with a daily intake of fish and omega-3 FAs:

	Fish intake								The intake of omega-3 FAs					
Variables	**Non/week**		**1-2/week**		**≥ 3/week**				**Total DHA (mg)**		**Total EPA (mg)**		**EPA + DHA (mg/day)**	
	n	%	n	%	n	*%*	(*df*) χ^2	*p*	Median	p	Median	p	Median	*p*
Age Mean						.								
Age (Y)										0.29		0.28		0.30
< 20	4	7.1	1	0.8	4	3.1	10.52	0.11	202		80		337	
20 – 30	27	48.2	51	42.9	57	44.9			165		121		286	
30 – 40	22	39.3	63	552.9	55	43.3			157		106		270	
> 40	3	5.4	4	3.4	11	8.7			220		155		375	
Educational level										0.75		0.90		0.79
Basic Education	5	8.9	9	7.6	14	11	1.833	0.76	185		126		309	
Secondary Education	22	39.3	53	44.5	58	45.7			164		107		268	
Degree or higher	29	51.8	57	47.9	55	43.3			157		107		268	
Occupational Status										0.81		0.73		0.77
Employed	18	32	32	26.9	41	32.3	0.981	0.61	167		122		290	
Unemployed	38	68	87	73.1	86	67.7			164		108		273	
Annual household income							2.661	0.61		0.32		0.27		0.31
Less than 500 OR	27	48.2	55	46.2	52	40.9			157		106		268	
500 OR – 1000 OR	20	35.7	50	42	52	49.9			169		118		289	
Above 1000	9	16.1	14	11.8	23	18.1			192		123		312	
Housing tenure							7.568	0.10		*0.07*		***0.05***		**0.05**
Rent house	16	28.6	31	26.1	31	*24.4*			164		104		250	
Homeowner	17	30.4	40	33.6	60	*47.2*			176		126		309	

Live with the husband or wife family	23	41.1	48	40.3	36	*28.3*			155		106		259	
Duration of Marriage							6.915	0.32		0.50		0.54		0.54
Less than two years	13	23.2	22	18.5	22	*17.3*			174		121		304	
Less than five years	17	30.4	20	16.8	24	*18.9*			155		108		269	
Less than ten years	16	28.6	44	37	48	*37.8*			161		115		273	
above ten years	10	17.9	33	27.7	33	*26*			166		118		279	
Planned pregnancy							.0217	0.89		0.61		0.23		0.43
Yes	36	64.3	80	67.2	86	*67.7*			166		119		282	
No	20	35.7	39	32.8	41	*32.3*			163		107		273	
Physical Activity							9.905	0.12		**0.05**		**0.05**		**0.04**
No physical activity	30	53.6	70	58.8	58	45.7			149		105		259	
30 minutes for at least two/ week	18	32.1	23	19.3	45	35.4			203		139		336	
30 minutes for at least 3/week	4	7.1	14	11.8	10	7.9			158		114		270	
30 minutes for at least 5/week	4	7.1	12	10.1	14	11			192		137		338	
History of Depression							6.257	**0.04**		**0.02**		**0.04**		**0.02**
Yes	7	12.5	18	15.1	7	55.5			110		91		195	
No	49	87.5	101	84.9	120	94.5			169		119		287	
Family history of depression							0.927	0.62		0.26		0.17		0.23
Yes	3	5.4	11	9.2	12	9.4			186		123		306	
No	53	94.6	108	90.8	115	90.6			164		114		274	
Parity							1.357	0.85		0.94		0.92		0.91
1	14	25	28	23.5	27	*21.3*			155		107		266	
2	15	26.8	25	21	32	*25.2*			186		122		309	

3	27	48.2	66	55.5	68	*53.5*			165		109		277	
Obesity BMI										0.26		0.29		0.28
Underweight	4	7.1	12	10.1	7	5.5	5.435	0.48	124		103		277	
Normal	12	21.4	30	25.2	33	26			203		139		344	
Overweight	17	30.4	38	31.9	31	24.4			165		107		273	
Obesity	23	41.1	39	32.8	56	44.1			168		108		271	
Obstetric complications previous pregnancy							4.094	0.66		0.28		0.33		0.31
Preeclampsia (PE)	2	3.6	3	2.5	3	2..4			157		95		252	
Miscarriage	11	19.6	31	26.1	34	26.8			165		119		282	
Preterm	3	5.4	3	2.5	9	7.9			215		156		371	
Mode of delivery in previous pregnancies							0.954	0.91		0.96		0.94		0.97
Normal Delivery (SVD)	30	53.6	62	52.1	72	56.7			164		114		281	
Caesarean section (CS)	15	26.8	37	31.1	34	31.1			164		106		272	
No previous pregnancies	11	19.6	20	16.8	21	16,8			170		127		292	

4.4.2 Fish and omega 3 FAs intake and depressive symptoms

Table 4-4 shows the association of fish and FAs intake of pregnant women at enrolment at 8-12 weeks of pregnancy in the study, with depression symptoms at weeks 8-12 and 24-28 of pregnancy. As presented in Table 4, there were significant differences in depressive symptoms during pregnancy among the study participants based on fish intake between 8 and 12 weeks of pregnancy. The highest incidence of depression symptoms among participants was in the group of women who did not eat fish at all (35.6% vs 11.3%; P <0.01) and among those who consumed approximately 1 to 2 portions of fish per week (58.9% vs 31.1%; P <0.01), both compared to the group without depressive symptoms. Pregnant women who consume three portions of fish per week or more were likelier to present with a low prevalence of depression symptoms (57.5% vs 5.6%; p<0.05). At the same time, as presented in Table 4-4, at 24 – 28 weeks of pregnancy, there were significant differences in depressive symptoms between participants in the study based on fish intake, with the highest prevalence of depression symptoms among participants being a group of women who did not eat fish at all (27.7% vs 15.9%; p<0.01), and between those who consumed about 1 to 2 portions of fish per week (50.6% vs 34.4%; p <0.01) both compared to the group without depressive symptoms. Pregnant women who consumed more than two portions of fish per week were likelier to present with a low prevalence of depression symptoms (49.7% vs 21.7%; p <0.05).

In terms of omega-3 FAs DHA and EPA intake at 8-12 weeks of pregnancy, as shown in Table 4-4, women with a high incidence of depressive symptoms (29.8%) revealed a significantly lower median [interquartile range (IQR)] intake of DHA and EPA (DHA,

medians (IQR) = 73.5 (38.7-132) vs 208 (145.7-275); p <0.01), (EPA, medians (IQR) = 66.5 (45.7-103) vs 143 (99-190.7); p <0.01), (omega-3 index, medians (IQR) = 147 (87.5-237.2) vs 348 (245-461.7); p <0.01) compared to the non-depressive groups (70.2%). Therefore, low omega-3 FAs intake may have a negative impact on the depression symptoms in the study sample. Meanwhile, at 24 to 28 weeks of pregnancy, as shown in Table 4, women with a high incidence of depressive symptoms (27.5%) revealed a significantly lower median [interquartile range (IQR)] intake of omega-3 FAs intake (DHA, median (IQR) = 110 (40-158) vs. 189 (109.5-267.5); p <0.01), (EPA, medians (IQR) = 81 (46-119) vs. 136 (83-179); p <0.01), (omega-3 index, medians (IQR) = 186 (88-271) vs. 325 (194.5-449.5); p <0.01), than to the non-depressive group (72.5%). Therefore, low omega-3 FAs intake may have a negative impact on the depression symptoms in the study sample.

Table 4-5 summarises the reports of unadjusted and adjusted logistic regression analyses for description of fish and omega-3 FAs intake results for women with and without antenatal depression and odds ratio (OR), confidence intervals and p values at 8 -12 weeks and 24 – 24 weeks of pregnancy. Women 8 to 12 weeks of gestation who did not eat fish were 3 times [AOR=3.133; 95% CI (1.509-9.968)] at risk of having prenatal depression. Similarly, women who did not eat fish at 24 – 28 weeks of pregnancy were four times [AOR= 4.004; 95% CI (1.908-8.401)] more likely to suffer from prenatal depression. However, women who consumed approximately 1 to 2 portions of fish per week were 1.6 times more likely than women who consume three portions of fish per week or more to suffer prenatal depression [AOR= 1.594, 95% CI (7.468-51.41)] between 8 and 12 weeks of pregnancy, and 3.37 times [AOR=3.374, 95% CI (1.786-6.376)]

between 24 and 28 weeks of pregnancy. This outcome does not alter after adjustment for possible confounders.

Regarding the DHA, EPA and omega-3 index, and after adjustment for possible confounders, significant relationships were observed between DHA, EPA and the omega-3 index and antenatal depression. From a univariate perspective, higher levels of DHA, EPA, and omega-3 index ($p < 0.01$) conferred lower odds of prenatal depression. Pregnant women with low DHA and EPA intake still had significantly higher odds of prenatal depression, with a relative risk nearly double that of women with higher intake of EPA and DHA at 8 and 12 weeks. Similar results were replicated at 24 and 24 weeks of pregnancy assessment.

Table 4-4 Association between fish and FAs intake with depression symptoms at week 8-12 and week 24-28 of pregnancy:

	at weeks 8-12 of pregnancy					at weeks 24-28 of pregnancy				
Variables	**Non-depressive** (EPDS ≤12) (n=212) (70.2%)		**Depressive** (EPDS ≥ 13) (n=90) (29.8%)			**Non-depressive** (EPDS ≤12) (n=189) (72.5%)		**Depressive** (EPDS ≥13) (n=83) (27.5%)		
Fish Consumption	n	%	n	%	*p*	n	%	n	%	*p*
Non/week	24	11.3	32	35.6	<.001	30	15.9	23	27.7	<.001
1-2/week	66	31.1	53	58.9	<.001	65	34.4	42	50.6	<.001
≥ 3/week	122	57.5	5	5.6	<.001	94	49.7	18	21.7	<.001
EPA+DHA Intake	**Median**	**Interquartile range**	**Median**	**Interquartile range**		**Median**	**Interquartile range**	**Median**	**Interquartile range**	
DHA (mg)	208	(145.7-275)	73.5	(38.7-132)	<.001	189	109.5-267.5	110	40-158	<.001
EPA (mg)	143	(99-190.7)	66.5	(45.7-103)	<.001	136	83-179	81	46-119	<.001
EPA + DHA (mg/day)	348	(245-461.7)	147	(87.5-237.2)	<.001	325	194.5-449.5	186	88-271	<.001

Table 4-5 Logistic regression analysis of risk factors for depression symptoms and fish and omega-3 FAs among pregnant Omani women in the study odds ratio (95% CI):

Variables	at week 8-12 of pregnancy				at week 24-28 of pregnancy			
	Unadjusted Model		Adjusted Model *		Unadjusted Model		Adjusted Model *	
	OR (95% C.I.)	*p*	OR (95% C.I.)	*P*	OR (95% C.I.)	*p*	OR (95% C.I.)	*p*
Above 3	-		-		-		-	
Non	3.133 (1.509-9.968)	<.001	3.809 (1.971-9.223)	<.001	4.004 (1.908-8.401)	.616	3.732 (1.732-8.038)	<.001
1-2	1.594 (7.468-51.41)	<.001	1.74 (6.62-4.538)	<.001	3.374 (1.786-6.376)	<.001	3.059 (1.587-5.898)	<.001
DHA (mg)	0.986 (0.982-0.990)	<.001	0.986 (0.982-0.990)	<.001	0.993 (0.991-0.996)	<.001	0.994 (0.991-0.996)	<.001
EPA (mg)	0.982 (0.976-0.987)	<.001	0.982 (0.976-0.988)	<.001	0.991 (0.986-0.995)	<.001	0.991 (0.987-0.995)	<.001
N 3	0.992 (0.990-0.994)	<.001	0.992 (0.990-0.994)	<.001	0.996 (0.994-0.998)	<.001	0.996 (0.995-0.998)	<.001

* a Variable(s) entered on step 1: Fish portion intake in one week by Category, Total DHA (mg) intake, Total EPA (mg) intake, Educational Level, Annual Household Income, History of Depression.

4.4.3 Fish and Omega 3 FAs intake and anxiety symptoms

Table 4-6 shows the relationship between fish and FAs intake on the part of pregnant women at enrolment in the study at 8 – 12 weeks of pregnancy, with anxiety symptoms at weeks 8 – 12 and 24-28. At 8 – 12 weeks, a higher prevalence of anxiety symptoms was observed among pregnant women who reported no fish intake (28% vs 15.4%; p <0.01) and among those who ate 1-2 portions of fish per week (50.7% vs 35.7%; p <0.01). Pregnant women who consume three portions of fish per week or more were likelier to present with a low prevalence of anxiety symptoms (48.9% vs 21.3%; p <0.01). Furthermore, at 24 – 28 weeks, a higher prevalence of anxiety symptoms was observed among pregnant women who reported no fish intake (25.4% vs 17.4%; p <0.01) and among those who ate 1-2 portions of fish per week (50.7% vs 35.7%; p <0.01). Patients with more than two fish portions per week were likelier to present with a low prevalence of anxiety symptoms (47.4% vs 23.9%; p <0.01).

As shown in Table 4-6, women at 8 to 12 weeks of pregnancy with a high prevalence of anxiety symptoms (24.8%) revealed a significantly lower median [interquartile range (IQR)] of DHA and EPA (DHA, median (IQR) = 110 (45-158) vs 183 (110-266); p <0.05), (EPA, median (IQR) = 84 (46-119) vs 126 (80-178); p <0.05), compared to the controlled group (75.2%). Therefore, low omega-3 FAs intake may negatively affect anxiety symptoms in the study sample. Meanwhile, women at 24 to 28 weeks of pregnancy with a high prevalence of anxiety symptoms (23.5%) revealed significantly lower median [interquartile range (IQR)] intake of DHA and EPA omega-3 FAs (DHA, median (IQR) = 124 (43-155) vs 183 (105-266); p <0.01), (EPA, median (IQR) = 90 (48-119) vs 126 (76-179); p <0.01), compared to the non-depressive group (76.5%). Therefore, a low intake

of omega-3 FAs may have negatively affected anxiety symptoms in the study sample. Table 4-7 summarised the reports of adjusted and unadjusted logistic regression analyses for the reports of fish and omega-3 FAs intake results for women with and without anxiety symptoms and the odds ratio (OR), confidence intervals, and p values at 8-12 weeks and 24-24 weeks of pregnancy. Women who did not eat fish at all were 4.1 times [AOR=4.162, 95% CI (1.96-8.841)] at 8-12 weeks of pregnancy and 2.8 times at 24 to 28 weeks of gestation [AOR=2.874; 95% CI (1.334 -6.194)] at risk of having a high rate of anxiety symptoms during pregnancy. On the other hand, women who consumed approximately 1 to 2 portions of fish per week were 3.2 times [AOR=3.255, 95% CI (1.678 to 8.841)] at 8-12 weeks of pregnancy and 2.8 times at 24 – 28 weeks of gestation [AOR=2.36; 95% CI 1.474-5.447] more likely to have a high rate of depression symptoms during pregnancy, after adjustment for possible confounders. Pregnant women with low DHA and EPA intake still had significantly higher odds of high rates of anxiety symptoms, with a relative risk nearly double that of those in the higher DHA and EPA intake categories at 8 and 12 weeks of pregnancy. Similar results were replicated at 24 and 28 weeks of pregnancy evaluation. There was a significant link between DHA, EPA and the omega-3 index with anxiety symptoms during pregnancy. From a univariate perspective, higher levels of DHA, EPA, and omega-3 index ($p < 0.01$) conferred lower odds of anxiety symptoms.

Table 4-6 Association between fish and FAs intake with anxiety symptoms at week 8-12 and week 24-28 of pregnancy:

	at weeks 8-12 of pregnancy					at weeks 24-28 of pregnancy				
	Non-anxiety (EPDS-3A ≤ 5) n=227(75.2%)		**Anxiety** (EPDS-3A ≥ 6) n=75 (24.8%)			**Non-anxiety** (EPDS-3A ≤ 5) n=201(76.6%)		**Anxiety** (EPDS-3A ≥ 6) n=71 (23.5%)		
Fish Consumption	n	%	n	%	***p***	n	%	n	%	***p***
Non/week	35	15.4	21	28	.000	35	17.4	18	25.4	.003
1-2/week	81	35.7	38	50.7	.000	71	35.3	36	50.7	.002
≥ 3/week	111	48.9	16	21.3	.000	95	47.4	17	23.9	.003
EPA+DHA intake	**Median**	**Interquartile range**	**Median**	**Interquartile range**	***p***	**Median**	**Interquartile range**	**Median**	**Interquartile range**	***p***
DHA (mg)	183	(110-266)	110	(45-158)	.000	183	105-266	124	43-155	.000
EPA (mg)	126	(80-178)	84	(46-119)	.000	126	76-179	90	48-119	.000
EPA + DHA (mg/day)	317	(191-445)	186	(100-278)	.000	317	178.5-449.5	213	100-271	.000

Table 4-7 Logistic regression analysis of risk factors for anxiety symptoms and fish and omega 3 FAs intake among pregnant Omani women in the study odds ratio (95% CI):

	at weeks 8-12 of pregnancy				at weeks 24-28 of pregnancy			
Variables	**Unadjusted Model**		**Adjusted Model ***		**Unadjusted Model**		**Adjusted Model**	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***p***	**OR (95% C.I.)**	***p***	**OR (95% C.I.)**	***p***
Above 3	-		-		-		-	
Non	4.162 (1.96-8.84)	<.001	3.859 (1.781-8.36)	<.001	2.874 (1.334 -6.194)	<.001	2.627(1.198-5.76)	<.001
1-2	3.255 (1.69-8.84)	<.001	2.939 (1.505-5.74)	<.001	2.833 (1.474-5.447)	<.001	2.613 (1.34-5.1)	<.001
DHA (mg)	0.986 (0.98-0.99)	<.001	0.996 (0.982-0.990)	<.001	0.994 (0.991-0.997)	<.001	.994 (991 - 997)	<.001
EPA (mg)	0.993(0.990-.996)	<.001	0.991 (0.987-0.996)	<.001	0.992 (0.988-0.996)	<.001	.993 (988 to 997)	<.001
EPA+ DHA (mg/day)	0.996 (0.94-0.99)	<.001	0.993 (0.990-0.996)	<.001	0.996 (0.995-0.998)	<.001	.997 (0.995-0.99)	<.001

* a Variable(s) entered on step 1: Fish portion intake in one week by Category, Total DHA (mg) intake, Total EPA (mg) intake, Educational Level, Annual Household Income, History of Depression,

4.4.4 Association between erythrocyte FAs levels and depression

Table 4-8 reports the median percentage of maternal erythrocyte FAs profile data for healthy and healthy and depressed controls between 8 controls between 8 and 12 weeks of gestation. Depressed pregnant women had significantly lower levels of arachidonic acid (20:4 n-6), total omega-6, docosahexaenoic acid (22:6 n-3) ($p = 0.034$), Lc ω6 docosapentaenoic acid (22:5 n-3), eicosapentaenoic acid (20:5 n-3) total omega-3 ($p= 0.005$), Lc ω3, omega-3 index ($p = 0.011$) and (AA+DHA)/MUFAs, but a higher omega-6/omega-3 ratio ($p=0.041$) than the non-depressed group.

Table 4-9 describes the FAs (absolute content) of the maternal erythrocyte profile for depressed and healthy control women at 8 – 12 weeks of pregnancy. Similarly, depressed pregnant women had a significantly lower content of arachidonic acid (20:4 n-6), Lc omega-6, docosapentaenoic acid (22:5 n-3), eicosapentaenoic acid (20:5 n-3), total omega-3 ($p = 0.001$), Lc omega-3, omega-3 index ($p = 0.024$), ratio AA / LA and (AA + DHA) / MUFAs, but a higher omega-6/omega-3 ratio ($p = 0.049$).

Table 4-10 reports the median percentage of maternal erythrocyte FAs profile data for healthy control women and depressed women between 24 – 28 weeks of pregnancy. When related to healthy women in the control group, depressed women are shown to have elevated palmitic acid (16:00), total saturated FAs and oleic acid (18:1 n-9), AA/DHA ratio and omega-6/omega-3 ratio ($p = <0.001$). Docosapentaenoic acid (22:5 n-3) ($p = 0.001$), eicosapentaenoic acid (20:5 n-3) ($p = 0.002$), total omega-3 ($p = <0.001$), omega-3 index ($p = <0.001$), (22:5/22:4 n-6) and (AA + DHA) / MUFAs ratios were significantly lower in pregnant women with prenatal depression. Table 4-11 shows the FAs (absolute content) of the maternal erythrocyte FAs profile for healthy control women at 24 – 28 weeks of pregnancy.

Although no significant differences were observed with regard to all omega-6 FAs and all MUFAs, a trend showed that women in the depressed group had lower eicosapentaenoic acid (20:5 n-3) ($p = 0.002$), docosahexaenoic acid (22:6 n-3) ($p = 0.004$), total omega-3 ($p = <0.001$), Lc omega-3, omega-3 index ($p = <0.001$), (22:5/22:4 n-6) and (AA+DHA)/MUFAs ratios ($p = 0.002$), with higher palmitic acid (16:00), total saturated FAs, AA / DHA, Lc omega-6/Lc omega-3 and omega-6/omega-3 ratios ($p = <0.001$) than those in the control group.

Table 4-8 Associations between maternal FAs levels (individual FAs % of total FAs in RBCs) and depressive symptoms at week 8-12 of pregnancy:

	Control (n=101)	**Depression (n=82)**	*p*
Fatty acid (% of total FAs in RBCs)	Median (Min-Max)	Median (Min-Max)	
14:00	0.93 (0.21-2.7)	1.02 (0.31-3.1)	0.497
16:00	24.12 (20.1-39.28)	26.3 (11-32.98)	0.126
18:00	13.17 (10.03-17.5)	13.515 (9.91-16.59)	0.488
20:00	0.78 (0.33-1.88)	0.865 (0.31-1.96)	0.315
22:00	1.82 (0.25-2.96)	1.39 (0.21-3)	0.442
24;00	3.62 (2.1-5.94)	3.84 (2.01-6.17)	0.222
∑SFA	45.51 (37.64-58.19)	46.035 (36.1-58.35)	0.296
16:1ω7	0.64 (0.1-1.99)	0.575 (0.21-2.07)	0.747
18:1ω9	10.49 (6.78-13.56)	11.02 (6.31-13.8)	0.214
18:1ω7	0.98 (0.48-1.57)	0.95 (0.51-2.2)	0.19
20:1ω9	0.74 (0.25-1.98)	0.79 (0.11-1.95)	0.76
22:1ω9	0.78 (0.13-2.17)	0.78 (0.21-1.93)	0.537
24:1ω9	3.26 (2.14-5.46)	3.28 (1.91-5.26)	0.491
∑MUFA	17.25 (12.99-21.97)	17.505 (13.1-21.95)	0.608
18:2ω6	6.52 (3.09-12.87)	7 (2.91-13.01)	0.891
18:3ω6	0.74 (0.16-2.67)	0.805 (0.31-2.38)	0.666
20:2ω6	0.72 (0.1-1.89)	0.675 (0.11-1.92)	0.671
20:3ω6	0.97 (0.26-2.61)	1.09 (0.41-2.66)	0.057
20:4ω6	13.8 (8-17.9)	12.5 (1-16.8)	**<.001**
22:2ω6	0.75 (0.12-1.86)	0.71 (0.11-1.82)	0.793
22:4ω6	1.43 (0.26-3.27)	1.51 (0.51-3.1)	0.108
22:5ω6	0.5 (0.16-1.98)	0.5 (0.11-1.59)	0.583
Lc ω6	19.29 (10.19-25.68)	18.07 (11.42-24.86)	**0.003**
∑ω6	26.53 (16.62-35.6)	25.46 (14.1-34.97)	**0.033**
18:3 ω3	0.62 (0.13-1.76)	0.6 (0.11-1.73)	0.535
20:3 ω3	0.5 (0.14-2.76)	0.43 (0.11-1.78)	0.107
20:5 ω3	1.02 (0.23-4.32)	0.75 (0.11-3.8)	**0.01**
22:5 ω3	1.6 (0.37-3.38)	1.16 (0.21-3.42)	**0.042**
22:6 ω3	5.9 (1.29-9.02)	3.85 (1.11-7)	**0.034**
Lc ω3	9.42 (3.14-14.56)	7.21 (3-13.44)	**0.007**
∑ω3	9.97 (4.37-15.2)	7.68 (3.21-14.33)	**0.005**
AA/LA	2.04 (0.7-4.32)	1.675 (0.71-4.84)	0.081
AA/DHA	2.56 (1.32-10.79)	3.065 (1.11-12.42)	0.673
ω6/ω3	2.65 (1.63-6.99)	3.375 (1.21-7.21)	**0.041**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	2.6409 (1.15-5.74)	0.086
22:5/22:4ω6	0.41 (0.06-3.33)	0.255 (0.01-1.49)	0.133
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	5 (1.81-10)	**0.011**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.915 (0.41-1.64)	**<.001**

Table 4-9 Associations between maternal FAs absolute quantities (mg/ml) and depressive symptoms at week 8-12 of pregnancy:

	Control (n=101)	Depression (n=82)	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.0125(0.0029-0.0378)	0.014 (0.005-0.0434)	0.538
16:00	0.3276(0.2613-0.5499)	0.34805 (0.2614-0.4617)	0.069
18:00	0.1778(0.1313-0.245)	0.18135 (0.1316-0.2309)	0.519
20:00	0.0106(0.0043-0.0263)	0.01175 (0.005-0.0274)	0.323
22:00	0.0239(0.0036-0.0412)	0.0195 (0.0029-0.042)	0.405
24;00	0.0491(0.0286-0.0798)	0.0499 (0.0268-0.0864)	0.305
∑SFA	0.6075(0.4893-0.8147)	0.63655 (0.5022-0.8168)	0.234
16:1ω7	0.0084(0.0013-0.0278)	0.0079 (0.0029-0.029)	0.758
18:1ω9	0.142(0.091-0.1899)	0.15 (0.0823-0.1932)	0.23
18:1ω7	0.0135(0.0062-0.0218)	0.01305 (0.0073-0.0308)	0.203
20:1ω9	0.0101(0.0033-0.0277)	0.01045 (0.0025-0.0254)	0.766
22:1ω9	0.0102(0.0018-0.0282)	0.0105 (0.0031-0.0264)	0.578
24:1ω9	0.0448(0.0279-0.0765)	0.04495 (0.0266-0.0736)	0.57
∑MUFA	0.2341(0.1818-0.3059)	0.2392 (0.1738-0.3073)	0.482
18:2ω6	0.0884(0.0433-0.1802)	0.091 (0.0406-0.1821)	0.887
18:3ω6	0.0099(0.0021-0.0374)	0.01085 (0.0042-0.0333)	0.7
20:2ω6	0.0095(0.0013-0.0265)	0.00935 (0.0017-0.0255)	0.665
20:3ω6	0.0129(0.0037-0.0365)	0.01485 (0.0058-0.0372)	0.065
20:4ω6	0.1518(0.088-0.1969)	0.1375 (0.077-0.1848)	**<.001**
22:2ω6	0.0098(0.0016-0.0242)	0.00995 (0.0017-0.0251)	0.791
22:4ω6	0.0199(0.0033-0.0425)	0.0202 (0.0077-0.0434)	0.104
22:5ω6	0.0055(0.0018-0.0218)	0.0055 (0.0014-0.0175)	0.583
Lc ω6	0.223 (0.1234-0.327)	0.2116 (0.1487-0.3342)	**0.006**
∑ω6	0.3203(0.1994-0.4597)	0.31265 (0.1791-0.443)	0.079
18:3 ω3	0.0068(0.0014-0.0194)	0.0066 (0.0018-0.019)	0.532
20:3 ω3	0.007(0.0018-0.0386)	0.00565 (0.0014-0.0231)	0.094
20:5 ω3	0.0137(0.003-0.0562)	0.00975 (0.0014-0.0532)	**0.02**
22:5 ω3	0.0176(0.0041-0.0372)	0.0128 (0.0032-0.0376)	**0.048**
22:6 ω3	0.078(0.0167-0.1263)	0.0539 (0.0153-0.098)	0.099
Lc ω3	0.1236 (0.043-0.2324)	0.0975 (0.0482-0.18632)	**0.013**
∑ω3	0.1292(0.053-0.2076)	0.10165 (0.0416-0.1878)	**0.01**
AA/LA	1.6848(0.5495-3.3974)	1.354 (0.5971-3.8035)	**0.047**
AA/DHA	2.0731(1.114-9.1301)	2.5933 (0.9483-10.5052)	0.811
ω6/ω3	2.4936(1.5128-6.871)	3.25165 (1.2764-7.0076)	**0.049**
Lc ω6/Lc ω3	1.8462 (1.328-5.843)	2.4018 (1.0637-5.25241)	0.126
22:5/22:4ω6	0.3232(0.0485-2.8205)	0.2057 (0.0491-1.2648)	0.09
ω3 Index (EPA+DHA)	0.0958(0.0289-0.1781)	0.07 (0.0243-0.14)	**0.024**
(AA+DHA)/MUFA	0.9281(0.4388-1.3093)	0.78955 (0.4278-1.3859)	**<.001**

Table 4-10 Associations between maternal FAs levels (individual FAs % of total FAs in RBCs and depressive symptoms at week 24-28 of pregnancy:

	Control (n=101)	**Depression (n=76)**	
Fatty acid (% of total FAs in RBCs)	Median (Min-Max)	Median (Min-Max)	*P*
14:00	0.93 (0.21-2.7)	1.35 (0.23-3.1)	0.254
16:00	24.12 (20.1-39.28)	27.82 (20.11-32.98)	**<.001**
18:00	13.17 (10.03-17.5)	13.53 (10.04-18)	0.867
20:00	0.78 (0.33-1.88)	0.75 (0.36-1.93)	0.214
22:00	1.82 (0.25-2.96)	1.6 (0.22-3.2)	0.794
24;00	3.62 (2.1-5.94)	3.05 (2.06-6.2)	0.093
∑SFA	45.51 (37.64-58.19)	48.03 (38.3-58.35)	**0.001**
16:1ω7	0.64 (0.1-1.99)	0.2 (0.22-1.95)	0.647
18:1ω9	10.49 (6.78-13.56)	11.285 (6.89-13.8)	**0.049**
18:1ω7	0.98 (0.48-1.57)	0.9 (0.52-1.95)	0.653
20:1ω9	0.74 (0.25-1.98)	0.55 (0.19-1.95)	0.634
22:1ω9	0.78 (0.13-2.17)	0.75 (0.24-1.93)	0.196
24:1ω9	3.26 (2.14-5.46)	3.8 (2.15-4.91)	0.366
∑MUFA	17.25 (12.99-21.97)	17.475 (13.79-22.65)	0.359
18:2ω6	6.52 (3.09-12.87)	7.8 (3.14-13.08)	0.299
18:3ω6	0.74 (0.16-2.67)	0.15 (0.31-2.38)	0.534
20:2ω6	0.72 (0.1-1.89)	0.55 (0.13-1.92)	0.64
20:3ω6	0.97 (0.26-2.61)	1.5 (0.5-2.77)	0.165
20:4ω6	13.8 (8-17.9)	13.45 (7.5-18.7)	0.1
22:2ω6	0.75 (0.12-1.86)	0.1 (0.13-1.82)	0.731
22:4ω6	1.43 (0.26-3.27)	1.6 (0.47-3.1)	0.071
22:5ω6	0.5 (0.16-1.98)	0.75 (0.11-1.59)	0.194
Lc ω6	19.29 (10.19-25.68)	18.78 (11.87-24.86)	0.134
∑ω6	26.53 (16.62-35.6)	26.025 (19.34-34.97)	0.592
18:3 ω3	0.62 (0.13-1.76)	0.95 (0.07-1.73)	0.246
20:3 ω3	0.5 (0.14-2.76)	0.5 (0.08-1.78)	0.083
20:5 ω3	1.02 (0.23-4.32)	0.95 (0.21-3.8)	**0.002**
22:5 ω3	1.6 (0.37-3.38)	1.6 (0.29-3.45)	0.125
22:6 ω3	5.9 (1.29-9.02)	3.5 (1.18-7)	**0.001**
Lc ω3	9.42 (3.14-14.56)	6.705 (3-13.44)	**<.001**
∑ω3	9.97 (4.37-15.2)	7.4 (3.28-14.33)	**<.001**
AA/LA	2.04 (0.7-4.32)	1.25 (0.57-4.84)	0.077
AA/DHA	2.56 (1.32-10.79)	3.25 (1.29-12.42)	**0.029**
ω6/ω3	2.65 (1.63-6.99)	3.65 (1.43-7.21)	**<.001**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	2.7222 (1.15-5.74)	**0.002**
22:5/22:4ω6	0.41 (0.06-3.33)	0.15 (0.06-1.49)	**0.023**
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	4.3 (1.87-10)	**<.001**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.8 (0.48-1.64)	**0.003**

Table 4-11 Associations between maternal FAs levels absolute quantities (mg/ml) and depressive symptoms at week 24-28 of pregnancy:

	Control (n=101)	Depression (n=76)	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.0125(0.0029-0.0378)	0.01425 (0.003-0.0403)	0.307
16:00	0.3276(0.2613-0.5499)	0.37995 (0.2614-0.4617)	**0.002**
18:00	0.1778(0.1313-0.245)	0.1832 (0.1316-0.234)	0.975
20:00	0.0106(0.0043-0.0263)	0.01175 (0.005-0.0251)	0.231
22:00	0.0239(0.0036-0.0412)	0.02045 (0.0029-0.0416)	0.7
24;00	0.0491(0.0286-0.0798)	0.05095 (0.0268-0.0864)	0.141
∑SFA	0.6075(0.4893-0.8147)	0.6587 (0.5022-0.8168)	**0.005**
16:1ω7	0.0084(0.0013-0.0278)	0.0085 (0.0029-0.0273)	0.702
18:1ω9	0.142(0.091-0.1899)	0.1517 (0.0895-0.1932)	0.108
18:1ω7	0.0135(0.0062-0.0218)	0.0133 (0.0073-0.0273)	0.497
20:1ω9	0.0101(0.0033-0.0277)	0.0112 (0.0025-0.0272)	0.702
22:1ω9	0.0102(0.0018-0.0282)	0.0091 (0.0031-0.0264)	0.194
24:1ω9	0.0448(0.0279-0.0765)	0.04485 (0.0282-0.0638)	0.335
∑MUFA	0.2341(0.1818-0.3059)	0.23805 (0.1835-0.3073)	0.522
18:2ω6	0.0884(0.0433-0.1802)	0.10735 (0.044-0.1802)	0.403
18:3ω6	0.0099(0.0021-0.0374)	0.01065 (0.0042-0.0333)	0.615
20:2ω6	0.0095(0.0013-0.0265)	0.009 (0.0018-0.0255)	0.613
20:3ω6	0.0129(0.0037-0.0365)	0.01405 (0.007-0.0359)	0.177
20:4ω6	0.1518(0.088-0.1969)	0.14795 (0.0825-0.2057)	0.1
22:2ω6	0.0098(0.0016-0.0242)	0.0099 (0.0017-0.0251)	0.677
22:4ω6	0.0199(0.0033-0.0425)	0.0214 (0.0061-0.0434)	0.083
22:5ω6	0.0055(0.0018-0.0218)	0.00525 (0.0012-0.0175)	0.193
Lc ω6	0.223 (0.1245-0.3253)	0.2205 (0.1423-0.3932)	0.158
∑ω6	0.3203(0.1994-0.4597)	0.3187 (0.2421-0.443)	0.7
18:3 ω3	0.0068(0.0014-0.0194)	0.00655 (0.0008-0.019)	0.246
20:3 ω3	0.007(0.0018-0.0386)	0.0049 (0.0011-0.0231)	0.069
20:5 ω3	0.0137(0.003-0.0562)	0.00935 (0.0027-0.0532)	**0.002**
22:5 ω3	0.0176(0.0041-0.0372)	0.0128 (0.0032-0.038)	0.134
22:6 ω3	0.078(0.0167-0.1263)	0.0441 (0.0153-0.098)	**0.004**
Lc ω3	0.1236 (0.04-0.2)	0.0815 (0.04-0.18)	**<.001**
∑ω3	0.1292(0.053-0.2076)	0.08935 (0.0416-0.1878)	**<.001**
AA/LA	1.6848(0.5495-3.3974)	1.3222 (0.4852-3.8035)	0.074
AA/DHA	2.0731(1.114-9.1301)	2.91495 (1.0104-10.5052)	**0.028**
ω6/ω3	2.4936(1.5128-6.871)	3.5593 (1.3918-7.0076)	**<.001**
Lc ω6/Lc ω3	1.8462 (1-5.8)	2.5 (1.06-5.25)	**0.003**
22:5/22:4ω6	0.3232(0.0485-2.8205)	0.178 (0.0491-1.2648)	**0.022**
ω3 Index (EPA+DHA)	0.0958(0.0289-0.1781)	0.05725 (0.0243-0.14)	**<.001**
(AA+DHA)/MUFA	0.9281(0.4388-1.3093)	0.83945 (0.4278-1.3859)	**0.002**

Table 4-12 and Table 4-13 summarise the unadjusted and adjusted univariate logistic regression analyses between the maternal FAs profile in the CR and the extent of antenatal depression among Omani women at 8-12 weeks and 24-28 weeks of pregnancy.

As shown in Table 4-12, several FAs were linked with a higher likelihood of experiencing prenatal depression among sample of pregnant Omani women. An unadjusted analysis found higher scores for arachidonic acid (20:4 n-6) (OR = 1.245, 95% CI 1.103 to1.406, p < .001), eicosapentaenoic acid (20:5 n-3) (OR = 1.315, 95% CI 1.023 to1.689, p = .03), docosahexaenoic acid (22:6 n-3) (OR = 1.17, 95% CI 1 to1.368, p = .049), total omega-3 FAs (OR = 1.145, 95% CI 1.038 to1.264, p = .007), omega-6/omega-3 ratio (OR = 0.774, 95% CI 0.618 to 0.97, p = 0.026), (22:5/22:4 n-6) ratio (OR = 2.397, 95% CI 1.061 to 5.418, p =0.036), lower omega-3 index (OR = 1.126, 95% CI 1.014 to 1.251, p= .027), (AA+DHA)/MUFAs ratio (OR = 9.766, 95% CI 2.546 to 37.456, p < .001). These were significantly linked to an increased risk of depression symptoms. These findings did not change after adjusting for potential confounders (annual income, educational level, and history of depression factors). Furthermore, adrenic acid (22:4 n-6) and eicosatrienoic acid (20:3 n-3) were significantly associated after adjustment for potential confounders.

As shown in Table 4-13, adjusted and unadjusted palmitic acid analyses (16:00), total saturated FAs, docosahexaenoic acid (22:6 n-3), eicosapentaenoic acid (20:5 n-3), total omega-3 FAs, omega-6 / omega-3 ratio, omega-6/omega-3 ratio, (22:5/22:4 n-6) ratio, omega-3 index and (AA+DHA)/MUFAs ratio were significantly linked with an increased risk of depression symptoms at week 24-28 of pregnancy. Furthermore, lignoceric acid (24:00) (OR = 0.72, 95% CI 0.519 to 0.998, p =0 .048), adrenic acid (22:4 n-6) (OR = 0.578 95% CI 0.366 to 0.913, p =0.019), and eicosatrienoic acid (20:3

n-3) (OR = 2.828, 95% CI 1.128 to 7.088, p =0.027), were significantly linked after adjusting for potential confounders (educational level and history of depression).

Table 4-12 Logistic regression analysis of risk factors for depression symptoms and FAs levels (individual FAs in total FAs of RBCs) at weeks 8-12 of pregnancy odds ratio (95% CI):

	at week 8-12 of pregnancy			
Fatty acids	**Unadjusted Model**		**Adjusted Model ***	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***p***
20:4ω6	1.245 (1.103-1.406)	**<.001**	1.266 (1.112-1.442)	**<.001**
22:4ω6	0.697 (0.465-1.044)	0.08	0.585 (0.373-0.918)	**0.02**
20:3 ω3	1.793 (0.83-3.874)	0.137	2.487 (1.049-5.896)	**0.038**
20:5 ω3	1.315 (1.023-1.689)	**0.032**	1.291 (0.987-1.688)	**0.041**
22:6 ω3	1.17 (1-1.368)	**0.049**	1.191 (1.003-1.413)	**0.046**
-∑ω3	1.145 (1.038-1.264)	**0.007**	1.151 (1.032-1.282)	**0.011**
ω6/ω3	0.774 (0.618-0.97)	**0.026**	0.762 (0.593-0.978)	**0.033**
22:5/22:4ω6	2.397 (1.061-5.418)	**0.036**	3.736 (1.339-10.421)	**0.012**
-ω3 Index (EPA+DHA)	1.126 (1.014-1.251)	**0.027**	1.131 (1.009-1.268)	**0.034**
(AA+DHA)/MUFA	9.76 (2.546-37.456)	**<.001**	10.725 (2.55 45.105)	**0.001**

Table 4-13 Logistic regression analysis of risk factors for depression symptoms and FAs levels (individual FAs in total FAs of RBCs) at weeks 24-28 of pregnancy odds ratio (95% CI):

	at week 24-28 of pregnancy			
Fatty acids	**Unadjusted Model**		**Adjusted Model ***	
	OR (95% C.I.)	***P***	**OR (95% C.I.)**	***p***
16:00	0.875 (0.809-0.946)	**<.001**	0.89 (0.819-0.966)	**0.006**
24;00	0.775 (0.574-1.046)	0.10	0.72 (0.519-0.998)	**0.048**
%-∑SFA	0.887 (0.828-0.95)	**<.001**	0.892 (0.829-0.96)	**0.002**
22:4ω6	0.671 (0.445-1.01)	0.056	0.578 (0.366-0.913)	**0.019**
20:3 ω3	2.214 (0.957-5.119)	0.063	2.828 (1.128-7.088)	**0.027**
20:5 ω3	1.479 (1.129-1.939)	**0.005**	1.476 (1.103-1.975)	**0.009**
22:6 ω3	1.278 (1.085-1.506)	**0.003**	1.285 (1.075-1.536)	**0.006**
-∑ω3	1.213 (1.091-1.349)	**<.001**	1.216 (1.082-1.366)	**<.001**
AA/DHA	0.839 (0.716-0.982)	**0.029**	0.838 (0.706-0.996)	**0.045**
ω6/ω3	0.662 (0.521-0.842)	**<.001**	0.658 (0.508-0.854)	**0.002**
22:5/22:4ω6	3.068 (1.239-7.6)	**0.015**	4.772 (1.542-14.764)	**0.007**
ω3 Index (EPA+DHA)	1.197 (1.07-1.339)	**0.002**	1.2 (1.063-1.355)	**0.003**
(AA+DHA)/MUFA	7.564 (1.979-28.91)	**0.003**	7.127 (1.713-29.656)	**0.007**

4.4.5 Association between erythrocyte FAs levels and anxiety

Table 4-14 reports the median percentage of maternal erythrocyte FAs profiles for anxiety and healthy control women at 8 –12 weeks of pregnancy. Women with high anxiety symptoms had significant low levels of cis-vaccenic acid (18:1 n-7), arachidonic acid (20:4 n-6), docosahexaenoic acid (22:6 n-3) (p = 0.01), eicosapentaenoic acid (20:5 n-3) (p = 0.037), total omega-3 FAs (p = 0.007), omega-3 index (p = 0.009) and (AA+DHA)/MUFAs (p = 0.021), but a higher palmitic acid (16:00) (p = 0.004), total saturated FAs (p = 0.039), and omega-6/omega-3 ratios (p = <0.014) than the control.

Table 4-15 summarises the FAs (absolute content) of the FAs profiles of maternal erythrocytes of anxious and healthy control women between 8 and 12 weeks of pregnancy. Similarly, women in the anxiety group had higher levels of palmitic acid (16:00) (p = 0.006), total saturated FAs (p = 0.032), and omega-6/omega-3 ratio (p = <0.016) than in the control group. Furthermore, women with antenatal depression had significantly lower content of docosahexaenoic acid (22:6 n-3) (p = 0.01), arachidonic acid (20:4 n-6), total omega-3 (p = 0.013), omega-3 index (p = 0.019), and the (AA+DHA)/MUFAs ratio.

Table 4-16 reports the median percentage of maternal erythrocyte FAs profile data for anxiety and healthy control women between 24 and 28 weeks of pregnancy. When related to healthy women, women with higher anxiety symptoms are shown to have elevated palmitic acid (16:00), lignoceric acid (24:00), total saturated FAs, oleic acid (18:1 n-9) total MUFAs and omega-6/omega-3 ratio (p = <0.001), arachidonic acid (20:4 n-6), osbond acid (22:5 n-6), dihomo-gamma-linolenic acid (20:3 n-6), eicosatrienoic acid (20 3 n-3) (p = 0.044), eicosapentaenoic acid (20:5 n-3) (p = 0.011), docosahexaenoic acid (22:6 n-3) (p = 0.09), total omega-3 (p = <0.001), Lc omega-3,

omega-3 index (p = <0.004), (22:5/22:4 n-6), and (AA + DHA) / MUFAs ratios, and Lc omega-6/Lc omega-3 were significantly lower in women with high anxiety symptoms.

Table 4-17 summarises the FAs (absolute content) of maternal erythrocyte FAs profile data for anxiety and healthy control women at 24 – 28 weeks of pregnancy. Compared to healthy women, women in the anxiety group have a high ratio of palmitic acid (16:00), lignoceric acid (24:00), total saturated FAs, oleic acid (18:1 n-9) and omega-6 / omega-3 (p = <0.001), dihomo-gamma-linolenic acid (20:3 n-6), arachidonic acid (20:4 n-6), osbond acid (22:5 n-6), eicosatrienoic acid (20:3 n-3) (p = 0.036), docosahexaenoic acid (22:6 n-3) (p = 0.029), total omega-3 (p = <0.002), Lc omega-3, omega-3 index (p = <0.008), (22:5/22:4 n-6), (AA+DHA)/MUFAs ratios and Lc omega-6/Lc omega-3, was significantly lower in women with high anxiety symptoms.

Table 4-14 Associations between maternal fatty acid levels (individual FAs% of individual FAs in RBCs) and anxiety symptoms at week 8-12 of pregnancy:

	Control (n=101)	**Anxiety (n=66)**	*p*
Fatty acid (% of total FAs in RBCs)	Median (Min-Max)	Median (Min-Max)	
14:00	0.93 (0.21-2.7)	0.995 (0.48-3.12)	0.884
16:00	24.12 (20.1-39.28)	27.82 (20.65-32.98)	**0.004**
18:00	13.17 (10.03-17.5)	13.305 (9.7-17.19)	0.469
20:00	0.78 (0.33-1.88)	0.855 (0.36-1.99)	0.505
22:00	1.82 (0.25-2.96)	1.41 (0.23-2.95)	0.328
24;00	3.62 (2.1-5.94)	3.815 (2.06-6.13)	0.147
∑SFA	45.51 (37.64-58.19)	46.26 (38.63-58.35)	**0.039**
16:1ω7	0.64 (0.1-1.99)	0.55 (0.22-1.95)	0.901
18:1ω9	10.49 (6.78-13.56)	11.08 (6.89-13.7)	0.255
18:1ω7	0.98 (0.48-1.57)	0.915 (0.52-1.8)	**0.042**
20:1ω9	0.74 (0.25-1.98)	0.715 (0.18-1.95)	0.353
22:1ω9	0.78 (0.13-2.17)	0.78 (0.23-1.95)	0.551
24:1ω9	3.26 (2.14-5.46)	3.315 (1.98-5.26)	0.94
∑MUFA	17.25 (12.99-21.97)	17.59 (12.77-21.95)	0.566
18:2ω6	6.52 (3.09-12.87)	7.625 (3.14-13.3)	0.332
18:3ω6	0.74 (0.16-2.67)	0.81 (0.31-2.21)	0.615
20:2ω6	0.72 (0.1-1.89)	0.58 (0.13-1.82)	0.315
20:3ω6	0.97 (0.26-2.61)	1.06 (0.38-2.66)	0.215
20:4ω6	13.8 (8-17.9)	13.55 (7-16.8)	**0.028**
22:2ω6	0.75 (0.12-1.86)	0.86 (0.13-1.82)	0.492
22:4ω6	1.43 (0.26-3.27)	1.345 (0.49-3.1)	0.615
22:5ω6	0.5 (0.16-1.98)	0.445 (0.12-1.59)	0.126
Lc ω6	19.29 (10.19-25.68)	18.46 (9.47-24.86)	**0.043**
∑ω6	26.53 (16.62-35.6)	25.985 (19.34-34.97)	0.284
18:3 ω3	0.62 (0.13-1.76)	0.56 (0.16-1.73)	0.254
20:3 ω3	0.5 (0.14-2.76)	0.36 (0.12-1.78)	0.137
20:5 ω3	1.02 (0.23-4.32)	0.875 (0.21-3.8)	**0.037**
22:5 ω3	1.6 (0.37-3.38)	1.16 (0.29-3.6)	0.242
22:6 ω3	5.9 (1.29-9.02)	4.1 (1.18-7)	**0.01**
Lc ω3	9.42 (3.14-14.56)	7.535 (3-12.89)	**0.014**
∑ω3	9.97 (4.37-15.2)	8.055 (3.28-13.97)	**0.007**
AA/LA	2.04 (0.7-4.32)	1.625 (0.53-4.74)	0.063
AA/DHA	2.56 (1.32-10.79)	3.065 (1.14-12.42)	0.195
ω6/ω3	2.65 (1.63-6.99)	3.275 (1.5-7.21)	**0.014**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	2.608 (1.17-5.74)	0.056
22:5/22:4ω6	0.41 (0.06-3.33)	0.25 (0.07-1.47)	0.064
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	5.545 (1.87-10)	**0.009**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	1.005 (0.47-1.64)	**0.021**

Table 4-15 Associations between maternal fatty acids absolute quantities (mg/ml) of pregnant women and anxiety symptoms at week 8-12 of pregnancy:

	Control (n=101)	**Anxiety (n=66)**	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	***p***
14:00	0.0125(0.0029-0.0378)	0.0136 (0.0062-0.0406)	0.902
16:00	0.3276(0.2613-0.5499)	0.3705 (0.2713-0.4617)	**0.006**
18:00	0.1778(0.1313-0.245)	0.17895 (0.1261-0.2309)	0.567
20:00	0.0106(0.0043-0.0263)	0.01155 (0.005-0.0259)	0.511
22:00	0.0239(0.0036-0.0412)	0.0195 (0.003-0.0384)	0.27
24;00	0.0491(0.0286-0.0798)	0.05135 (0.0268-0.0858)	0.171
∑SFA	0.6075(0.4893-0.8147)	0.64305 (0.5022-0.8168)	**0.032**
16:1ω7	0.0084(0.0013-0.0278)	0.0072 (0.0029-0.0273)	0.9
18:1ω9	0.142(0.091-0.1899)	0.1514 (0.0895-0.1899)	0.266
18:1ω7	0.0135(0.0062-0.0218)	0.01235 (0.0073-0.0234)	**0.047**
20:1ω9	0.0101(0.0033-0.0277)	0.0097 (0.0025-0.0254)	0.353
22:1ω9	0.0102(0.0018-0.0282)	0.0109 (0.0031-0.0273)	0.561
24:1ω9	0.0448(0.0279-0.0765)	0.04555 (0.0257-0.0736)	0.891
∑MUFA	0.2341(0.1818-0.3059)	0.23865 (0.166-0.3073)	0.59
18:2ω6	0.0884(0.0433-0.1802)	0.1022 (0.044-0.1802)	0.351
18:3ω6	0.0099(0.0021-0.0374)	0.0109 (0.0042-0.0309)	0.657
20:2ω6	0.0095(0.0013-0.0265)	0.00815 (0.0018-0.0255)	0.343
20:3ω6	0.0129(0.0037-0.0365)	0.01415 (0.0049-0.0372)	0.198
20:4ω6	0.1518(0.088-0.1969)	0.14905 (0.077-0.1848)	**0.028**
22:2ω6	0.0098(0.0016-0.0242)	0.01165 (0.0017-0.0251)	0.515
22:4ω6	0.0199(0.0033-0.0425)	0.01855 (0.0063-0.0434)	0.588
22:5ω6	0.0055(0.0018-0.0218)	0.0049 (0.0013-0.0175)	0.129
Lc ω6	0.223 (0.1235-0.3893)	0.2158 (0.1321-0.3932)	0.06
∑ω6	0.3203(0.1994-0.4597)	0.31925 (0.2421-0.443)	0.469
18:3 ω3	0.0068(0.0014-0.0194)	0.0062 (0.0018-0.019)	0.255
20:3 ω3	0.007(0.0018-0.0386)	0.0049 (0.0015-0.0231)	0.088
20:5 ω3	0.0137(0.003-0.0562)	0.01135 (0.0027-0.0532)	0.056
22:5 ω3	0.0176(0.0041-0.0372)	0.0128 (0.0032-0.0396)	0.408
22:6 ω3	0.078(0.0167-0.1263)	0.0574 (0.0153-0.098)	**0.028**
Lc ω3	0.1236 (0.0454-0.2123)	0.0991 (0.0423-0.1745)	**0.02**
∑ω3	0.1292(0.053-0.2076)	0.10435 (0.0416-0.1826)	**0.013**
AA/LA	1.6848(0.5495-3.3974)	1.37415 (0.4453-3.7275)	0.06
AA/DHA	2.0731(1.114-9.1301)	2.52985 (0.967-10.5052)	0.223
ω6/ω3	2.4936(1.5128-6.871)	3.27405 (1.3918-7.0076)	**0.016**
Lc ω6/Lc ω3	1.8462 (1-5.8)	2.3375 (1.06-5.25)	0.063
22:5/22:4ω6	0.3232(0.0485-2.8205)	0.2067 (0.0584-1.2397)	0.062
ω3 Index (EPA+DHA)	0.096(0.0289-0.1781)	0.0758 (0.0243-0.14)	**0.019**
(AA+DHA)/MUFA	0.9281(0.4388-1.3093)	0.87015 (0.4169-1.3859)	**0.016**

Table 4-16 Associations between maternal fatty acids levels (individual FAs % of total FAs in RBCs) and anxiety symptoms at week 24-24 of pregnancy:

	Control (n=101)	**Anxiety (n=66)**	
Fatty acid (% of total FAs in RBCs)	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.93 (0.21-2.7)	0.995 (0.48-2.9)	0.547
16:00	24.12 (20.1-39.28)	26.63 (18-32.98)	**0.023**
18:00	13.17 (10.03-17.5)	13.65 (10.04-17.16)	0.366
20:00	0.78 (0.33-1.88)	0.83 (0.36-1.89)	0.371
22:00	1.82 (0.25-2.96)	1.43 (0.25-2.95)	0.181
24;00	3.62 (2.1-5.94)	4.03 (2.06-6.2)	**0.008**
∑SFA	45.51 (37.64-58.19)	46.74 (38.63-58.35)	**0.026**
16:1ω7	0.64 (0.1-1.99)	0.545 (0.12-1.95)	0.53
18:1ω9	10.49 (6.78-13.56)	11.43 (6.89-13.89)	**0.001**
18:1ω7	0.98 (0.48-1.57)	0.975 (0.52-1.95)	0.507
20:1ω9	0.74 (0.25-1.98)	0.84 (0.19-1.94)	0.945
22:1ω9	0.78 (0.13-2.17)	0.65 (0.17-1.89)	0.094
24:1ω9	3.26 (2.14-5.46)	3.435 (2.15-5.26)	0.823
∑MUFA	17.25 (12.99-21.97)	17.745 (13.79-22.65)	**0.039**
18:2ω6	6.52 (3.09-12.87)	7.98 (3.14-12.95)	0.231
18:3ω6	0.74 (0.16-2.67)	0.785 (0.24-2.38)	0.815
20:2ω6	0.72 (0.1-1.89)	0.625 (0.13-1.92)	0.471
20:3ω6	0.97 (0.26-2.61)	1.18 (0.5-2.77)	**0.006**
20:4ω6	13.8 (8-17.9)	13.35 (7.8-18.7)	**0.043**
22:2ω6	0.75 (0.12-1.86)	0.77 (0.13-1.79)	0.969
22:4ω6	1.43 (0.26-3.27)	1.555 (0.47-3.1)	0.052
22:5ω6	0.5 (0.16-1.98)	0.435 (0.14-1.59)	**0.044**
Lc ω6	19.29 (10.19-25.68)	18.745 (12.9-24.86)	0.167
∑ω6	26.53 (16.62-35.6)	25.915 (19.34-34.97)	0.69
18:3 ω3	0.62 (0.13-1.76)	0.56 (0.07-1.73)	0.066
20:3 ω3	0.5 (0.14-2.76)	0.35 (0.08-1.78)	**0.044**
20:5 ω3	1.02 (0.23-4.32)	0.75 (0.21-3.8)	**0.011**
22:5 ω3	1.6 (0.37-3.38)	1.16 (0.17-3.29)	0.051
22:6 ω3	5.9 (1.29-9.02)	3.535 (1.18-7)	**0.009**
Lc ω3	9.42 (3.14-14.56)	6.66 (3-13.09)	**0.003**
∑ω3	9.97 (4.37-15.2)	7.24 (3.28-13.52)	**0.001**
AA/LA	2.04 (0.7-4.32)	1.72 (0.6-4.84)	**0.031**
AA/DHA	2.56 (1.32-10.79)	3.285 (1.28-12.42)	0.18
ω6/ω3	2.65 (1.63-6.99)	3.77 (1.5-7.21)	**0.001**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	2.6868 (1.09-5.74)	**0.004**
22:5/22:4ω6	0.41 (0.06-3.33)	0.2 (0.06-1.49)	**0.007**
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	4.895 (1.87-10)	**0.004**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.93 (0.48-1.64)	**<.001**

Table 4-17 Associations between maternal fatty acids absolute quantities (mg/ml) and anxiety symptoms at week 24-28 of pregnancy:

	Control (n=101)	**Anxiety (n=76)**	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	*P*
14:00	0.0125 (0.0029-0.0378)	0.0135 (0.0062-0.0388)	0.594
16:00	0.3276 (0.2613-0.5499)	0.3582 (0.234-0.4617)	**0.03**
18:00	0.1778 (0.1313-0.245)	0.186 (0.1348-0.231)	0.406
20:00	0.0106 (0.0043-0.0263)	0.01145 (0.005-0.0246)	0.382
22:00	0.0239 (0.0036-0.0412)	0.02 (0.0033-0.0384)	0.167
24;00	0.0491 (0.0286-0.0798)	0.0562 (0.0268-0.0819)	**0.01**
∑SFA	0.6075 (0.4893-0.8147)	0.64535 (0.5022-0.8168)	**0.029**
16:1ω7	0.0084 (0.0013-0.0278)	0.0074 (0.0016-0.0273)	0.507
18:1ω9	0.142 (0.091-0.1899)	0.1556 (0.0895-0.1932)	**0.004**
18:1ω7	0.0135 (0.0062-0.0218)	0.01325 (0.0073-0.0273)	0.403
20:1ω9	0.0101 (0.0033-0.0277)	0.011 (0.0025-0.0272)	0.924
22:1ω9	0.0102 (0.0018-0.0282)	0.0088 (0.0022-0.0264)	0.105
24:1ω9	0.0448 (0.0279-0.0765)	0.0476 (0.0282-0.0736)	0.777
∑MUFA	0.2341 (0.1818-0.3059)	0.24065 (0.1891-0.3073)	0.067
18:2ω6	0.0884 (0.0433-0.1802)	0.10665 (0.044-0.1802)	0.263
18:3ω6	0.0099 (0.0021-0.0374)	0.01055 (0.0031-0.0333)	0.882
20:2ω6	0.0095 (0.0013-0.0265)	0.00875 (0.0018-0.0255)	0.456
20:3ω6	0.0129 (0.0037-0.0365)	0.01545 (0.007-0.0372)	**0.006**
20:4ω6	0.1518 (0.088-0.1969)	0.14685 (0.0858-0.2057)	**0.043**
22:2ω6	0.0098 (0.0016-0.0242)	0.01045 (0.0017-0.0251)	0.999
22:4ω6	0.0199 (0.0033-0.0425)	0.02155 (0.0061-0.0434)	0.052
22:5ω6	0.0055 (0.0018-0.0218)	0.00475 (0.0015-0.0175)	**0.045**
Lc ω6	0.2232 (0.1232-0.3087)	0.2177 (0.1536-0.3098)	0.221
∑ω6	0.3203 (0.1994-0.4597)	0.321 (0.2421-0.443)	0.883
18:3 ω3	0.0068 (0.0014-0.0194)	0.0062 (0.0008-0.019)	0.067
20:3 ω3	0.007 (0.0018-0.0386)	0.0049 (0.0011-0.0231)	**0.036**
20:5 ω3	0.0137 (0.003-0.0562)	0.01015 (0.0027-0.0532)	**0.017**
22:5 ω3	0.0176 (0.0041-0.0372)	0.0128 (0.0019-0.0362)	0.058
22:6 ω3	0.078 (0.0167-0.1263)	0.0487 (0.0153-0.098)	**0.029**
Lc ω3	0.1236 (0.0421-0.2093)	0.0849 (0.0487-0.1783)	**0.005**
∑ω3	0.1292 (0.053-0.2076)	0.0891 (0.0416-0.1781)	**0.002**
AA/LA	1.6848 (0.5495-3.3974)	1.36195 (0.5097-3.8035)	**0.035**
AA/DHA	2.0731 (1.114-9.1301)	2.60675 (1.0085-10.5052)	0.173
ω6/ω3	2.4936 (1.5128-6.871)	3.5593 (1.3918-7.0076)	**0.001**
Lc ω6/Lc ω3	1.8462 (1.8653-5.563)	2.5637 (1.8632-5.25937)	**0.007**
22:5/22:4ω6	0.3232 (0.0485-2.8205)	0.16035 (0.0468-1.2648)	**0.007**
ω3 Index (EPA+DHA)	0.0958 (0.0289-0.1781)	0.066 (0.0243-0.14)	**0.008**
(AA+DHA)/MUFA	0.9281 (0.4388-1.3093)	0.797 (0.4278-1.3859)	**<.001**

Table 4-18 and Table 4-19 summarise the unadjusted and adjusted univariate logistic regression analyses between the maternal FAs profile in RBC and anxiety symptoms among Omani women at 8-12 and 24-28 weeks of pregnancy. As shown in Table 4-18, an unadjusted analysis found higher scores for palmitic acid (16:00) (OR = 0.881, 95% CI 0.81 to 0.958, p < .003), arachidonic acid (20:4 n-6) (OR = 1.156, 95% CI 1.018 to 1.312, p < .025), docosahexaenoic acid (22:6 n-3) (OR = 1.208, 95% CI 1.019 to 1.432, p = .03), total omega-3 FAs (OR = 1.154, 95% CI 1.036 to1.286, p = .009), omega-6/omega-3 ratio (OR = 0.7551, 95% CI 0.587 to 0.961, p = 0.023), (22:5/22:4 n-6) ratio (OR = 3.04, 95% CI 1.154 to 8,004, p =0.024), omega-3 index (OR = 1.136, 95% CI 1.014 to 1.273, p = .028), (AA+DHA)/MUFAs ratio (OR = 4.626, 95% CI 1.211 to 17.669, p < .025), and that this was significantly linked to an increased risk of anxiety symptoms. These findings did not change after adjusting for possible confounders (educational level, history of depression, and whether pregnancy was planned). Furthermore, after adjusting for potential confounders, osbond acid (C22:5n-6) and eicosatrienoic acid (20:3 n-3) were significantly associated. The exception was for total saturated FAs, with the result being insignificant when adjusting for potential confounders (p = 0.078). Table 4-19, shows that several FAs were associated with higher anxiety symptoms among Omani women at weeks 24-28 of pregnancy. After adjusting for possible confounders (educational level and history of depression), palmitic acid (16:00), lignoceric acid (24:00), total saturated FAs, arachidonic acid (20:4 n-6) and osbond acid (C22:5n-6) were significantly linked with the risk of increased anxiety symptoms at weeks 24-28 of pregnancy. Interestingly, this study found for the first time that oleic acid (18:1n-9), total MUFAs, and dihomo-gamma-linolenic acid (C20:3n-6) were associated with greater anxiety symptoms among Omani women. Furthermore, adjusted and unadjusted analyses showed that almost

all omega-3 FAs were significantly linked with a high risk of anxiety symptoms at weeks 24-28 of pregnancy. Anxiety symptoms were also related to omega-6/omega-3 ratios (22: 5 / 22: 4) n-6 and (AA+DHA)/MUFAs ratios with omega-3 indexes after adjustment for confounders.

Table 4-18 Logistic regression analysis of risk factors for anxiety symptoms and FAs levels (individual FAs % of total FAs in RBCs) at week 8-12 of pregnancy with the study odds ratio (95% CI):

	At weeks 8-12 of pregnancy			
Fatty acids	**Unadjusted Model**		**Adjusted Model ***	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***P***
16:00	0.881 (0.81-0.958)	**0.003**	0.9 (0.823-0.984)	**0.021**
SFA	0.915 (0.85-0.984)	**0.017**	0.933 (0.863-1.008)	0.078
20:4ω6	1.156 (1.018-1.312)	**0.025**	1.173 (1.026-1.341)	**0.02**
22:5ω6	2.081 (0.803-5.393)	0.131	3.062 (1.015-9.243)	**0.047**
20:3 ω3	1.947 (0.839-4.519)	0.121	2.667 (1.04-6.837)	**0.041**
22:6 ω3	1.208 (1.019-1.432)	**0.03**	1.227 (1.02-1.475)	**0.03**
∑ω3	1.154 (1.036-1.286)	**0.009**	1.163 (1.034-1.307)	**0.012**
ω6/ω3	0.751 (0.587-0.961)	**0.023**	0.735 (0.563-0.96)	**0.024**
22:5/22:4ω6	3.04 (1.154-8.004)	**0.024**	5.724 (1.641-19.97)	**0.006**
ω3 Index (EPA+DHA)	1.136 (1.014-1.273)	**0.028**	1.143 (1.011-1.293)	**0.033**
(AA+DHA)/MUFA	4.62 (1.211-17.669)	**0.025**	5.094 (1.224-21.199)	**0.025**

Table 4-19 Logistic regression analysis of risk factors for anxiety symptoms and FAs levels (individual FAs % in total FAs in RBCs) at week 24-28 of pregnancy in the study odds ratio (95% CI):

	At weeks 24-28 of pregnancy			
Fatty acids	**Unadjusted Model**		**Adjusted Model ***	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***P***
16:00	0.91 (0.838-0.988)	**0.025**	0.92 (0.841-0.999)	**0.049**
24;00	0.655 (0.478-0.897)	**0.008**	0.607 (0.435-0.847)	**0.003**
∑SFA	0.907 (0.844-0.974)	**0.007**	0.907 (0.842-0.977)	**0.011**
18:1ω9	0.743 (0.608-0.909)	**0.004**	0.727 (0.586-0.901)	**0.004**
∑MUFA	0.824 (0.694-0.978)	**0.027**	0.828 (0.69-0.993)	**0.042**
20:4ω6	1.138 (1.006-1.287)	**0.039**	1.141 (1.002-1.299)	**0.047**
22:5ω6	2.523 (0.947-6.722)	0.064	3.567 (1.189-10.703)	**0.023**
20:3 ω3	2.858 (1.103-7.406)	**0.031**	3.274 (1.181-9.075)	**0.023**
22:6 ω3	1.22 (1.028-1.447)	**0.023**	1.213 (1.011-1.455)	**0.038**
∑ω3	1.201 (1.076-1.341)	**0.001**	1.191 (1.059-1.339)	**0.004**
ω6/ω3	0.674 (0.527-0.861)	**0.002**	0.691 (0.532-0.897)	**0.005**
22:5/22:4ω6	3.648 (1.338-9.943)	**0.011**	5.475 (1.684-17.803)	**0.005**
ω3 Index (EPA+DHA)	1.163 (1.035-1.307)	**0.011**	1.154 (1.02-1.305)	**0.023**
AA+DHA)/MUFA	9.722 (2.334-40.49)	**0.002**	8.907 (2.033-39.022)	**0.004**

4.5 Discussion

This is the first study to evaluate the associations between maternal FAs intake, maternal erythrocyte FAs profile, and anxiety and depressive symptoms in sampled pregnant women in Oman.

4.5.1 Fish and omega 3 FAs intake

The study shows low fish intake in sample of pregnant Omani women. Around 20% of the study sample reported not eating fish, and about 39% reported limiting their fish consumption to less than two portions per week. Only 20 pregnant women (6.6%) reported taking dietary supplements containing omega-3 FAs during pregnancy. Like fish intake, the daily maternal median intake of DHA and EPA (165 mg/d and 116.5 mg/d, respectively) was less than the nutritional recommendations.

There is limited evidence on the intake of fish and omega-3 FAs among pregnant women, and what there is provides various estimates. Consistent with our findings, a recent study in Norway aimed at evaluating fish intake in pregnant women revealed that only 29.1% of women followed the national recommendation to eat seafood 2 to 3 times a week, and 77% reportedly took an omega-3 FAs supplement (705). In terms of EPA and DHA intake, a study in Belgium (706) found that pregnant women consumed a regular of 120 mg of EPA and 150 mg of DHA daily. 24.6% of them also took omega-3 FAs supplements, similar to our findings. Furthermore, a German study that included 55 pregnant women reported that they ate one portion of fish per week, 20% taking an omega-3 FAs supplement (707).

Similar to our findings, numerous studies indicate that fish consumption in pregnant women in many countries worldwide is often below recommended levels (165). This is an issue, as fish is an essential source of nutrients, including vitamins, protein, zinc,

iodine, iron and omega-3 FAs (121), all of which are essential for the development of the fetus (174,179). These findings suggest that the recommendation to limit fish consumption among pregnant women to avoid exposure to methylmercury has led to a harmful reduction in fish intake (174,177).

4.5.2 Fish and omega-3 FAs intake on depressive and anxiety symptoms

The findings of this study show a significant association between fish and omega-3 FAs intake and depressive and anxiety symptoms at weeks 8-12 and 24-28 of pregnancy. Significant associations remained even after controlling for potential confounders. These findings support other previous evidence that suggests that fish and omega-3 FAs intake may offer protection against antenatal depression, specifically DHA, which is associated with increased depressive and anxiety symptoms in pregnant women (260–262). Furthermore, several cohort studies indicate that a high omega-6 / omega-3 FAs ratio of - 9: 1 in early pregnancy is associated with increased depressive symptoms (263). A ratio of 1:1 to 3:1 omega-6/omega-3 FAs should be the target ratio for health. In contrast, four additional studies have reported that there is no significant link between omega-3 FAs intake and depression in pregnant women (259,264–266). However, factors, for example, age, alcohol use, physical activity and smoking, can influence the relationship between fish and FAs intake and the absorption and distribution of FAs in the body, including integration into peripheral tissues (132).

4.5.3 Fatty acid composition of erythrocytes with depressive and anxiety symptoms

The current cohort study showed that women with antenatal depression or high anxiety symptoms during pregnancy had depleted erythrocyte levels of arachidonic acid (20:4 n-6), total omega-6 FAs, docosahexaenoic acid (22:6 n-3) docosapentaenoic acid

(22:5 n-3), eicosapentaenoic acid (20:5 n-3) total omega-3, omega-3 index and (AA+DHA)/MUFAs, but a higher omega-6/omega-3 ratio compared to controlled group.

The outcome of this study is consistent with those of other studies that show a significant relationship between FAs levels in erythrocytes and prenatal depression and revealed that low levels of omega-3 FAs, specifically low concentrations of DHA or higher n-6 / n-3 ratios in erythrocytes, correspond to a high rate of antenatal depression (256,263,267–273). However, these findings have yet to be replicated in other studies (274,275).

Omega-3 FAs have anti-inflammatory effects, prevent oxidative stress, and impact brain physiology (708). Although omega-6 FAs are often considered pro-inflammatory, a high omega-6/omega-3 ratio can adversely affect health (133)). Furthermore, an imbalance between FAs in the omega-6/omega-3 ratio can trigger an overproduction of pro-inflammatory cytokines containing IFNγ, IL-1, IL-6, and TNFα. It can also lead to alterations in the number and function of serotonin (5-HT) and dopamine receptors (DR-2) (709), thus adding to the causes of depression.

Pregnancy is associated with higher demand for omega-3 FAs (710), particularly during the third trimester, to meet biological needs and ensure fetal development (711). However, research on the link between omega-3 FAs intake (261,265,712,713) and erythrocyte levels (256,263,274,712,714) with prenatal depression and anxiety have produced inconsistent results.

The current study shows that women with a high rate of antenatal depression or anxiety symptoms had decreased erythrocyte levels of the omega-3 index compared to healthy women. The findings are constant with those of other findings. A higher rate

of postpartum depression has been linked with a lower omega-3 index in the last stages of pregnancy (256). Furthermore, Da Rocha et al, (263) reported that in a group of Brazilian women, a low omega-3 index during the 28th week of pregnancy is associated with greater postnatal depression. A Belgian cohort of 72 healthy women indicated that an omega-3 index level below 5% meant that they were five times at risk of experiencing postpartum depression compared to a group with an omega-3 index level of 5% or higher (268). There is growing concern about the role of the omega-3 index in mental health. (715–718). Research suggests that a low omega-3 index may be linked with a higher risk of CHD mortality (719,720). Currently, there is no defined optimal omega-3 index to reduce the chances of depression. However, our findings support the theory of the role of the omega-3 index in depression, as previously proposed by Milte and colleagues (717).

Regarding arachidonic acid (20:4 n-6), total omega-6 FAs, AA and depression have a negative association, although previous theories indicate that AA increases oxidative stress (721). AA can activate brain cannabinoid receptors, reduce oxidative stress, and potentially alleviate depression symptoms (722). AA and DHA are significant components of brain neural cells (121). AA plays a crucial role in maintaining membrane fluidity, neurotransmission, and synaptic plasticity, specifically in the hippocampus (723). Thus, AA helps to preserve neural function more than it causes neural damage due to oxidation (724). AA is vital for central nervous system development, placental function, immune system regulation, and neurotransmission (121). While the amount of DHA is higher in neurons, astrocytes have a greater proportion of AA than DHA, with the ratio of AA/DHA = 4.8. Astrocytes play a pivotal role in brain function, including protecting against peroxidation and maintaining the brain's immune system. AA levels in astrocytes are worth considering in terms of the

impact on the mental health during pregnancy (725). The reduction in AA levels could be why the benefits of high levels of EPA and DHA supplements are not as evident in several clinical trials that aimed to evaluate the effect of omega-3 supplements on pre- and post-natal depression (268).

Interestingly, palmitic acid (16:00), lignoceric acid (24:00), and total SFAs were found to be elevated in women with higher symptoms of anxiety and depression. Studies on the association between FAs levels and depression have focused on omega-3 FAs, specifically DHA and EPA. Other FAs may be linked to depression, but few studies have explored this association. One involved a case-control study that demonstrated that FAs levels in plasma total saturated FAs (SFAs) and monounsaturated FAs (MUFAs) were higher in women with major depressive disorder than to healthy women (726). Furthermore, a study in Japan showed that higher palmitic acid levels (16:00) were associated with elevated depressive symptoms (724). However, none of these studies examined the relationship with antenatal depression. The consumption of SFAs, specifically palmitic acid (PA), can cause inflammation and oxidative stress in adipocytes. This can result in the buildup of reactive oxygen species, which can lead to symptoms of depression (726). Proinflammatory cytokines and C-reactive adipose tissue proteins can circulate throughout the body through the blood or lymphatic systems (727). Cytokines can reach the brain and cause increased neural susceptibility by triggering overactivity of the hypothalamic-pituitary-adrenal (HPA) axis, a feature observed in depressive disorders (721,728). In addition, inflammation can cause an increase in oxidative stress, leading to alterations in neuronal membrane stability and the transmission of catecholamines in the brain. These changes can ultimately affect an individual's susceptibility to depression (369,701).

4.5.4 Clinical trials

The effective doses of omega-3 supplements for an anti-depressive effect in prenatal and postnatal women are unclear. For example, researchers have not observed a significant reduction in depressive symptoms among breastfeeding mothers using 0.2 g/d of DHA supplement for four months. The outcome of the (289) trial could be due to the use of a lower dose of DHA than the required dose (0.3 g/d) for lactating women, as recommended by several groups to completely replace DHA in the brain (290,291). Furthermore, RCTs that examined the promised benefits of omega-3 FAs in antenatal and postnatal depression did not indicate significant effects when using supplements with a low ratio of EPA to DHA (283,285–288) or a pure DHA supplement (292), or a mixture of DHA and EPA (1 g/D to 4.37g/D) in terms of maternal depression symptoms (255,283,284). Alternatively, clinical trials using pure EPA or a high ratio of EPA to DHA (1 to 3.5 g per d) for approximately two months during pregnancy showed a significant reduction in depressive symptoms among pregnant women (276–279) and postpartum depression (281,282).

In a meta-analysis aiming to evaluate the efficacy of omega-3 FAs supplementation in postnatal depression (729), the researchers did not report any beneficial effects. The same result was obtained in a review by Gould et al, (26). These reviews did not differentiate between the dosage, type, or timing of omega-3 FAs supplementation. According to Hsu et al, (117), taking EPA supplements can alleviate depression symptoms during pregnancy and childbirth. Similarly, DHA supplements can reduce depression symptoms during pregnancy. Another review by Wojcicki et al, (730) shows that earlier high dosages of EPA/DHA in pregnancy are linked with lower rates of perinatal depression. Additionally, a meta-analysis suggested that omega-3 FAs

supplementation benefits women with severe depression symptoms during pregnancy (494).

Reviews disagree on the possible effect of omega-3 FAs supplementation on reducing depression symptoms. However, it is obvious that the inconsistencies in the results of these studies are due to variations in the study design and issues pertinent to the optimal dosage. The clinical trials presented to date contrast in terms of critical methodological aspects, such as depression diagnosis, intervention time, monotherapy versus augmentation, type of placebo, doses, and percentages of DHA and EPA in supplements (117). Recently, two main factors have been suggested to explain the discrepancies between the efficacy of EPA compared to DHA and its effectiveness as a treatment, but not as a prevention agent, in diagnosing depressive disorders (232). The ideal dose and ratio of components in omega-3 FAs supplements for mental health disorders have received little attention. To date, there are no agreed guidelines. A meta-analysis tested the hypothesis that EPA is the practical component in omega-3 treatment of major depressive episodes and found that EPA in proportions greater than 60% of DHA, with a ceiling of around 2,000 mg of EPA over DHA, positively affected depression outcomes (731).

4.5.5 Erythrocyte Fatty acid percentages vs absolute quantity

In the current study, FAs levels were determined by the percentage of FAs in total FAs and absolute quantities. The implications of these varied approaches have yet to be addressed. Researchers have found significant differences between the two methods (732,733,733,734). FAs proportions may be used to assess the significance of a specific FAs set compared to the overall FAs level. On the contrary, absolute quantities are used to measure FAs independently without considering the levels of other FAs

(131,132,733). FAs can increase in proportion or decrease the relative proportion of other FAs in total, even if the absolute quantities remain the same (131,132).

Membrane fatty acid composition is functionally linked with membrane protein architecture. This is why and how changes in membrane lipid composition alter function (130). Research has indicated that cell membrane function depends on the balance reached by percentage and particular FAs, including SFAs, MUFAs, and LCPUFAs (735–737). For example, a study conducted on FAs levels in the brain of females with schizophrenia than in healthy women showed that the postmortem hippocampal membranes of schizophrenic patients had a 20% lower DHA content than those of the control group (736). It has been recommended that FAs are expressed as absolute quantities (733), but others prefer the percentage of FAs as a reliable means of assessment to predict nutritional status and health outcomes (132,732). Numerous studies have used percentages instead of quantities when studying the relationship between erythrocyte FAs profile and prenatal depression (256,257,268,270) or with pregnancy outcomes (329,738–741).

4.5.6 Strengths and limitations

The strength of the present analysis is the longitudinal assessment of symptoms of anxiety and depression in women at two points during pregnancy. Studies on prenatal anxiety and depression symptoms involving repetitive assessment and suitable statistical analyses are scarce. The entry and removal methods for the logistic regression model used in this study are effective as they involve repeated observations and monitor variations in FAs levels and depressive symptoms throughout pregnancy. Another strength of the study is that it is based on FAs levels in erythrocytes rather than relying on proxy measures such as supplements or self-reported FFQ. Erythrocytes are suggested to be a more consistent measure of long-

term nutritional intake than plasma, which can reflect recent dietary intake (742). Using non-fasting blood samples further supports its accuracy.

There are a few possible limitations to this research study. First, the assessment of omega-3 FAs intake is based on FFQ. As with other dietary assessment methods, these questionnaires allow for possible incorrect recall and inaccurate expectations regarding food portion sizes regarding dietary intake. However, this was limited in this study by using food model samples to estimate food portion sizes and validating omega-3 FAs FFQ with FAs levels in erythrocytes. Second, all participants in the study had a similar possibility of being misclassified with respect to their results and exposure status. This could lead to bias in the power of the link between outcome and exposure. Due to its observational nature, unassessed outstanding factors may confound the work; for example, fish intake could function as a proxy for a healthy lifestyle. Therefore, potential confounders with seafood intake or depression were recorded and used as adjustment variables in the data analysis. Furthermore, antenatal depression is linked to various risk factors that fall under psychological, social, and biological categories (743,744), and distinguishing between these factors can be challenging. Certain aspects may have a more direct correlation with the condition, while others may act as causal factors. Adjusting for a nonrelevant factor in a multifactorial disease could create new biases. Third, we decided to use a convenient non probabilistic sampling procedure in a sample of pregnant women with enough time and willingness to answer all interview questions. Non-randomized sampling restricts generalizing about the population, as some of its features may not be well represented in the sample.

4.6 Conclusion

The outcomes of the prospective cohort study show a significant association between fish and omega-3 FAs intake and depressive and anxiety symptoms among sample of pregnant Omani women at weeks 8-12 and 24-28 of pregnancy. Women with antenatal depression or high anxiety symptoms during pregnancy had a lower erythrocyte concentration of arachidonic acid (20:4 n-6), total omega-6 FAs, docosahexaenoic acid (22:6 n-3), docosapentaenoic acid (22:5 n-3), eicosapentaenoic acid (20:5 n-3) total omega-3, omega-3 index and (AA+DHA)/MUFAs, but a higher omega-6/omega-3 ratio compared to healthy women.

Clinical trials on the beneficial role of omega-3 FAs supplements during pregnancy on prenatal and postnatal depression could be an essential step towards cementing the observed association. Such a study would clarify the potential benefit of omega-3 FAs supplementation for both mothers and children and provide the necessary evidence for a significant public health initiative. Fish and seafood intake and omega-3 FAs supplementation are highly recommended for women during pregnancy to ensure the well-being of both the mother and fetus.

Chapter 5: Fish and Omega-intake and FAs levels and pregnancy outcome

5.1 Introduction

Long-chain omega-6 and omega-3 FAs are critical parts of tissue lipids, particularly cell membrane phospholipids (745). For example, AA can be generally found in all cellular membranes. It represents up to 15% of the total FAs in most tissue phospholipids (746) and about 40% in placental inner membranes (747). In contrast, DHA has a more specific tissue distribution as very high levels can be found in the cerebral cortex, retina, and testis (748). High levels of DHA are found in all mammals (109,749), which provides an initial indication as to the importance of DHA (121). Maintaining sufficient levels of DHA during pregnancy is imperative for ensuring the child's optimal cognitive and visual development (750,751). The synthesis of DHA and AA in the body is often inadequate, and whereas AA is freely obtainable in normal Western dietary intakes (752,753), DHA is comparatively restricted (754). However, studies indicate that women are at risk of AA and DHA deficiency during pregnancy and postpartum (121).

Optimal nutritional intake of DHA during pregnancy might improve the maternal DHA levels which are essential for child neurodevelopment (755,756), theoretically benefiting the fetus (757). Furthermore, women with an adequate intake of oily fish sees an improvement in maternal DHA levels and supports the DHA supplied to the fetuses (758). In addition, a dietary intake of a low omega-6/omega-3 FAs ratio is essential for physiological functions and metabolic pathways relevant to fetal development (136,759,760). However, in a Western diet, the nutritional intake changes over time, which triggers an extreme alteration in the omega-6/omega-3 FAs

ratio from 1:1 to 19 – 30:1 (136), which affects placental development and endorses the pathogenesis of various chronic disorders (133).

The amount of omega-3 FAs stored in tissue in pregnant women decreases throughout pregnancy (249,253). Therefore, fish intake and omega–3 FAs supplementation is regularly suggested during pregnancy (761). Omega-3 FAs supplementation during pregnancy benefits the development of the fetus and the mother's health (762).

A study in the Faroe Islands suggests that a high oily fish intake during pregnancy may result in a longer gestation age and improved pregnancy outcomes (293). Many studies, including clinical trials, have investigated the link between omega-3 FAs acids and preterm birth. Despite this, some studies have failed to confirm the positive impact of omega-3 FAs on preterm birth (140,294–298). Nevertheless, others indicate that high omega-3 FAs levels and intake in pregnant women could potentially lead to a longer gestation age and a decrease in LBW per preterm birth (73,141,299–301) a reduction in neonatal death and stillbirth rate (141), an enhancement in neurocognitive growth (304) and the prevention of children’s allergies (302–304).

Omega-3 FAs can lower inflammation and oxidative stress in GDM women (316). According to research, women with GDM tend to have insufficient omega-3 FAs and vitamin levels (312–314). The taking of omega-3 FAs supplements during pregnancy may improve pregnancy outcomes and lower the risk of GDM (315). Furthermore, PE in pregnant women is linked to low Omega-3 FAs levels (337,338). One observational study suggests that a pregnant woman's risk of PE can be reduced by 24% with a 1% increase in omega-3 FAs levels (339).

Overall, although numerous studies have examined the links between a mother's AA and DHA levels and their impact on pregnancy and neonatal outcomes, the findings have been inconsistent.

5.2 Aim

The overall aims of the chapter are to examine whether there is an association between seafood or omega-3 PUFA intake and: (i) the erythrocytes level during pregnancy; and maternal and newborn outcomes among sample of pregnant Omani women in the Al-Buraimi governorate, Oman.

In addition, the biomagnification process of FAs in maternal and cord erythrocytes from maternal circulation through the placenta to fetal circulation will be assessed. Further, the percentage and absolute of total fatty acids will be measured and related to the estimated fish and Omega-3 fatty acid intake.

5.3 Methods

This prospective cohort study recruited 300 pregnant Omani women receiving maternal care at Al Buraimi Hospital, Oman. The Ethics and Research Committee Board of the Oman Ministry of Health approved the study protocol on 1 July 2019 (MoH/CSR/19/9668) (The ethical approval is attached in Appendix 4). Participants were recruited from the antenatal clinics of Al Buraimi Hospital and the Primary Health Center in Al Buraimi, which serves the local community of the Al-Buraimi Governorate . In summary, patient electronic medical records were reviewed to obtain antenatal, intrapartum, and postpartum data. Maternal characteristics and complications were evaluated using general health questionnaires. Pregnancy and neonatal outcome records were collected from mothers and their offspring and used to achieve the aims

and objectives of the study. The methods used, including inclusion and exclusion criteria, were described in detail in Chapter 2.

Fasting blood samples (5ml) were collected in a preservation tube containing ethylene diamine tetraacetic acid (BD Vacutainer® EDTA tubes) at 8-12 weeks of gestation. The erythrocyte membrane and plasma FAs were isolated and stored in the -80°C freezer until needed for analysis. In this research project, lipid extraction was done from maternal and cord erythrocyte samples following the Folch et al. approach, as shown in the Materials and Methods section in Chapter 2. After the FAs were extracted, the FAMEs were separated using GC. The separated compounds were then loaded into an autosampler ampule and repeatedly injected into the Agilent GC-7890A device equipped with an MS-Premium low bleed column (60 m x 0.32 mm ID, 0.25 µm film, BP20) (SGE Analytical Science, Australia) range with a maximum temperature of 260°C. A computer-based data chromatography system was used to determine the peak areas in the sample in terms of percentage values (Agilent Open LAB chromatography data system, Scientific Software Inc., San Ramon, CA). The peak areas were calculated based on the percentage of total peaks identified.

5.3.1 Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 21, developed by IBM Corp., Chicago, Illinois, USA. Descriptive statistics were used to describe pregnant women's socioeconomic, demographic, and psychological data. For continuous variables, the results were expressed as means, and standard deviations (SD), while categorical variables were presented as frequencies and percentages.

The relationship Between fish intake during pregnancy (fish portion per week) and the estimated amount of the median daily intake of omega-3 FAs among pregnant women who completed all three assessments points were assessed using the one-way ANOVA on ranks (Kruskal-Wallis) analysis. The relationship between fish and EPA and DHA FAs intake and level and pregnancy outcomes among the study samples were matched. For continuous variables, the Mann-Whitney U test was employed, while. For categorical variables, the Chi-squared test was used.

Logistic regression analysis tests were set to evaluate 95% confidence intervals (CIs) and odds ratios (ORs). Both tests explored the association between fish and FAs intake, levels, and pregnancy outcomes. Outcomes were stated as odds ratio (OR) with 95% confidence interval (95% CI) or linear regression coefficients (β) with 95% confidence interval (95% CI). The detailed statistical analysis was described in detail in (Chapter 2).

5.4 Results

5.4.1 Fish and omega 3 FAs intake and pregnancy and neonatal outcomes

By the end of the study, 242 sample of pregnant Omani women had completed the three assessment points (recruitment measurements at 8 – 12 weeks of pregnancy, measures at 24 – 28 weeks of pregnancy and measurements at childbirth, including pregnancy and neonatal outcomes). The details of the sociodemographic features, clinical characteristics, and the pregnancy and newborn outcomes of the women who completed the three assessment points are provided in Chapter 3. The fish intake by portion per week, and the median (interquartile range) nutritional intake of DHA and EPA, are shown in Table 5-1. The fish consumption of 1-2 portions per week and more than two portions of fish per week at 8 – 12 weeks of pregnancy was reported by 98 of the pregnant women (40.5%). Only 46 (19%) of the study sample reported that they

did not eat fish at all. The median (interquartile range) dietary intake of DHA and EPA FAs at 8 – 12 weeks of pregnancy is shown in Table 5-1. The daily maternal median intake of DHA and EPA at 8 – 12 weeks of pregnancy was estimated to be about 157 mg/d and 108.5 mg/d, respectively. The median total daily intake of omega-3 index was 268 (mg/day). As described in Table 1, out of 242 women, only 15 (6.1%) reported taking dietary supplements containing omega-3 FAs during pregnancy, while 45 (18.3%) reported taking nutritional supplements, including vitamins, during pregnancy. Table 5-2 shows the relationship between fish consumption per week at 8-12 weeks of pregnancy and daily median nutritional intake of DHA and EPA omega-3 FAs. Significant differences in the median intake of DHA, EPA, and total omega-3 index existed across the fish consumption categories (p=<.001). For example, as was expected, the median intake of DHA (264 mg/d), EPA (178 mg/d) and omega-3 index (444 mg/d) were all higher in the case of pregnant women who consumed more than three fish portions per week. Furthermore, pregnant women who took no or 1-2 fish per week were expected to have a lower daily median intake of DHA and EPA FAs.

Table 5-1 Distribution of daily intake of fish and omega 3 FAs and dietary supplements N 242:

Fish intake during pregnancy	**n**	**%**
Non/week	46	19
1-2/week	98	40.5
≥ 3/week	98	40.5
Omega-3 fatty acids	**Median**	**Interquartile Range**
Total DHA (mg)	157	(72-249)
Total EPA (mg)	108.5	(65-169)
Total n-3 EPA + DHA (mg/day)	268	(129-421)
Dietary supplements containing omega-3 PUFAs during pregnancy	15	6.1
Dietary supplements containing vitamins during pregnancy	45	18.3

Table 5-2 Relationship between fish intake (fish portion/week) and the estimated amount of the median daily intake of EPA and DHA N 242.

Daily intake of Omega-3 fatty acids	Fish Consumed (portion/wk)			
	Non/week	1-2/week	≥ 3/week	
	Median	Median	Median	*p*
Total DHA (mg)	31	124	264	<.001
Total EPA (mg)	43.5	97	178	<.001
Total n-3 EPA + DHA (mg/day)	74	213	442	<.001

Table 5-3 shows the association linking fish intake by portion per week and median nutritional intake of EPA and DHA with pregnancy and neonatal outcomes among the study participants. The median intake of DHA and omega-3 index were significantly linked with the incidence of GDM among women in the study (p = 0.031) and (p = 0.05), respectively. None of the pregnancy and neonatal outcome variables, PE and spontaneous preterm birth, were significantly associated with the nutritional intake of fish or omega-3 FAs.

Table 5-4 summarised the relationships involving omega-3 FAs intake and neonatal birth outcomes (gestational duration (days)) and customised birthweight centile in unadjusted linear regression analyses. The unadjusted linear regression analyses indicate significant positive associations between gestational period by days and daily intake of DHA (p = 0.05). The DHA level was linked with .016 days longer gestational age (β = .016 days/unit DHA; 95% CI -.033 to .0001). Furthermore, In the unadjusted linear regression analyses (Table 5-4), each variable of the total daily intake of EPA and DHA omega-3 FAs intake were significantly positively associated with customised birthweight centile (EPA: p = 0.006, DHA: p = 0.047 and omega-3 index p = 0.003).

Table 5-3 Association between fish and omega-3 FAs intake and pregnancy and neonatal outcomes of the Omani pregnant women in the study N (242):

		Fish Intake								Omega-3 Fatty Acids Intake					
Variables		**Non/week**		**1-2/week**		**≥ 3/week**				**DHA (mg)**		**EPA (mg)**		**N 3 index (mg/day)**	
Spontaneous preterm		n	%	n	%	n	*%*	(*df*) χ^2	*p*	Median	p	Median	p	Median	*p*
Preterm	16	2	1.9	6	5.6	8	8.5	0.082	0.960	164	0.201	121	0.325	283	0.233
Healthy control	104	12	12.1	36	36.4	56	55.5			220		143		375	
GDM															
Women with GDM	59	8	7.2	21	20.6	30	31.1	0.2	0.905	183	**0.031**	126	0.134	325	**0.05**
Healthy control	104	12	12.8	36	36.4	56	54.9			220		143		375	
Preeclampsia															
Women with PE	13	3	1.7	5	4.6	5	6.8	1.773	0.412	149	0.204	88	0.161	251	0.181
Healthy control	104	12	20	36	36.4	56	54.2			220		143		375	

Table 5-4 Liner regression investigated the association between maternal fatty acids intakes with gestational duration (days) and customised birthweight centile:

	β	95%CI	p		β	95%CI	p
Gestational Duration (days)				**Customised birthweight centile**			
Total DHA	.016	.033 to .0001	**.05**	Total DHA	.047	.017 to .076	**0.002**
Total EPA	.016	.039 to .007	.179	Total EPA	.057	0.016 to 0.097	**0.006**
n-3 FAs	.009	.018 to .001	.08	n-3 FAs	.027	.009 to 0.044	**0.003**

5.4.2 FAs Biomagnification

Table 5-5 and Table 5-6 describe the variations in FAs proportions and absolute content (mg/mL) found in the erythrocytes of paired analysis of maternal and fetal blood circulation. The ratio of total and individual saturated FAs, specifically, palmitic acid (16:00), stearic acid (18:00), lignoceric acid (24:00), and total saturated FAs, were significantly lower in the maternal than in the blood cord erythrocytes. Interestingly, the lowest median percentage for the maternal detected was for stearic acid (13.285 vs 16.985 %) and lignoceric acid (3.825 vs 4.88 %) in the cord blood (Figure 5-1and Figure 5-2). Conversely, monounsaturated FAs were lower in cord blood compared to maternal erythrocytes. For example, the nervonic acid (24:1n-9) and oleic acid (18:1n-9) median proportions in the cord blood were notably lower than in the maternal erythrocyte (3.01vs 3.385 %) and (8.18 vs 11.27 %) respectively. Furthermore, the proportion of the total MUFAs was higher in the maternal erythrocyte than in the erythrocyte in cord blood (17.78 vs 14.55 %).

In terms of omega-6 FAs, the maternal levels of the parent precursor linoleic acid (18:2 n-6) were more elevated than erythrocytes in cord blood ($p = <0.001$). In contrast, the arachidonic acid (20:4 n-6) and its precursors, adrenic acid (22:4n-6) and DPA (C22:5n-6) were significantly elevated in cord erythrocytes than in maternal erythrocytes ($p = <0.001$). Adrenic acid (22:4n-6) and arachidonic acid (20:4 n-6) were increased from maternal blood to cord blood by 13.55 % to 14.87 % and 1.82 % to 2.53 %, respectively.

The situation is exciting with regard to the omega-3 FAs due to the smaller precursor found in the maternal circulation containing a smaller precursor that is further reduced in the fetus. For example, α-LA (18:3 n-3) exhibited a concentration of 0·51% in

maternal erythrocytes, which further decreased significantly to 0·23% in the cord blood.

As for the LC omega-3 FAs, eicosapentaenoic acid (20:5 n-3) was 0.98 % in the maternal circulation but experienced a significant reduction to 0.56 % in the fetus cord blood ($p < 0·01$). The maternal proportions of docosapentaenoic acid (22:5 n-3) were similarly low at 1.23 %, that is further reduced in passage across the placenta at 0.76% ($p < 0·01$). In contrast to arachidonic acid (20:4 n-6), the biomagnification of docosahexaenoic acid (22:6 n-3) observed in the fetal erythrocytes was comparatively small (4.11% to 4.4%) ($p = 0.034$). The 22:5/22:4n-6 and (AA+DHA)/MUFA ratios were significantly higher in the fetal erythrocytes than in the maternal erythrocytes ($p = <0.001$). No difference was observed in the omega-3 index preparation.

The examination of the correlations between maternal and fetal FAs levels in erythrocytes is described in Table 5-5 and Table 5-6. FAs proportions in maternal and fetal blood erythrocytes of oleic acid (C18:1 n-9), gondoic acid (20:1 n-9), total MUFAs, arachidonic acid (20:4 n-6), total omega-6 FAs, docosahexaenoic acid (22:6 n-3), total omega-3 FAs, AA/DHA, omega-6/omega-3 ratio and the omega-3 index, were all positively correlated (Table 5-5).

Except for C18:0, saturated FAs were not constantly increased in favour of the fetus in erythrocytes. Similar to the proportions of FAs in fetal erythrocytes, nervonic acid (24:1n-9), oleic acid (18:1n-9) and the total MUFAs appear to be consistently lower than in maternal erythrocytes (Figure 5-3). The levels of linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) was notably more elevated in maternal erythrocytes than in fetal circulation ($p = <0.001$). In contrast, the absolute quantities of arachidonic acid (20:4 n-6) and its precursor DPA (C22:5n-6) were consistently lower in maternal

erythrocytes than in fetal. As explained in Table 5-6, the eicosapentaenoic acid (20:5 n-3) content was also significantly decreased in the fetal RBCs and, consistently, the docosahexaenoic acid (22:6 n-3) content was similar between fetal and maternal erythrocytes. The absolute quantities of the AA/LA, AA/DHA, 22:5/22:4n-6, omega-6/omega-3 and (AA+DHA)/MUFA ratios were significantly higher in fetal erythrocytes than in the maternal erythrocytes ($p = <0.001$). We observed similar correlations in terms of FAs between maternal and fetal RBCs in absolute quantities to those observed when the FAS were expressed asproportions. Stearic acid (18:00), oleic acid (C18:1 n-9), gondoic acid (20:1 n-9), arachidonic acid (20:4 n-6), total omega-6 FAs, docosahexaenoic acid (22:6 n-3), and total omega-3 FAs, were all positively correlated (Table 5-6.). Moreover, the omega-3 index, AA/DHA and omega-6/omega-3 ratio were also positively correlated in maternal and fetal RBCs measured in absolute quantities.

Table 5-5 Biomagnification process of relative proportions of fatty acids in RBCs (%):

	Maternal (n=172)	**Cord (n=172)**	*Wilcoxon*	*Spearman*	
Fatty acid profile (%)	Median (Min-Max)	Median (Min-Max)	*p*	r_s	*p*
14:00	0.975 (0.23-2.77)	0.885 (0.38-2.3)	0.002	-0.003	0.969
16:00	27.37 (20.11-32.87)	30.37 (21.67-36.9)	<.001	0.002	0.984
18:00	13.285 (10.12-17.5)	16.985 (11.16-21.6)	<.001	0.098	0.101
20:00	0.815 (0.19-1.99)	1.02 (0.12-2.45)	0.007	-0.026	0.731
22:00	1.005 (0.2543-2.76)	1.2 (0.21-1.91)	0.632	-0.072	0.351
24:00	3.825 (2.06-6.17)	4.88 (2.27-5.96)	<.001	0.009	0.902
∑SFA	46.407 (37.64-56.92)	54.299 (41.24-66.05)	<.001	-0.009	0.906
16:1ω7	0.62 (0.1-1.95)	0.76 (0.23-1.98)	0.002	0.033	0.667
18:1ω9	11.27 (7.9-14.02)	8.18 (2.97-11.71)	<.001	**.141***	**0.033**
18:1ω7	0.96 (0.52-1.95)	1.3425 (0.42-2.38)	<.001	0.053	0.487
20:1ω9	0.755 (0.1-1.98)	0.58 (0.05-1.74)	<.001	**-.126***	**0.049**
22:1ω9	0.715 (0.14-1.95)	0.63 (0.06-1.98)	0.016	-0.111	0.146
24:1ω9	3.385 (2.15-5.35)	3.01 (1.6-6.54)	<.001	0.034	0.662
∑MUFA	17.78 (13.01-22.652)	14.55 (8.37-19.96)	<.001	**.133***	**0.041**
18:2ω6	8.55 (5.02-12.87)	2.82 (1.05-5.96)	<.001	0.131	0.088
18:3ω6	0.745 (0.11-2.67)	0.725 (0.16-1.73)	0.01	0.056	0.469
20:2ω6	0.71 (0.1-1.97)	0.53 (0.1-1.8)	<.001	0.005	0.952
20:3ω6	1.28 (0.65-2.765)	1.875 (0.6-3.9)	<.001	0.093	0.225
20:4ω6	13.55 (8-17.9)	14.87 (8.02-20.5)	<.001	**.147***	**0.027**
22:2ω6	0.74 (0-1.94)	0.58 (0.01-1.69)	<.001	-0.073	0.341
22:4ω6	1.82 (0.92-3.272)	2.53 (1-4.88)	<.001	-0.021	0.784
22:5ω6	0.3145 (0.16-0.68)	0.8 (0.29-1.95)	<.001	-0.031	0.685
∑ω6	27.461 (20.54-35.596)	25.245 (16.43-33.81)	<.001	**.179***	**0.019**
18:3 ω3	0.515 (0.12-1.2)	0.235 (0.03-0.62)	<.001	-0.025	0.745
20:3 ω3	0.3 (0.14-0.88)	0.44 (0.07-1.88)	<.001	0.028	0.714
20:5 ω3	0.98 (0.2-3.7)	0.56 (0.07-1.98)	<.001	0.054	0.483
22:5 ω3	1.23 (0.17-3.382)	0.76 (0.05-1.96)	<.001	0.026	0.736
22:6 ω3	4.11 (1.18-7)	4.4 (2.15-7.76)	0.034	**.196****	**0.01**
∑ω3	8.3 (3.79-15.197)	6.69 (2.86-11.4)	<.001	**.228****	**0.003**
AA/LA	1.424 (0.6993-3.1743)	5.34 (2.1409-13.3143)	<.001	0.037	0.629
AA/DHA	2.90 (1.2836-12.4153)	3.4193 (1.2037-7.4)	0.238	**.246****	**0.001**
ω6/ω3	3.39 (1.4332-7.2067)	3.77 (1.608-11.1552)	0.108	**.239****	**0.002**
22:5/22:4ω6	0.17 (0.0553-0.6186)	0.30 (0.0727-1.4333)	<.001	-0.111	0.147
ω3 Index (EPA+DHA)	5.77 (1.87-10.7)	5.13 (2.47-9.38)	0.112	**.209****	**0.006**
(AA+DHA)/ MUFA	0.9675 (0.484-1.5121)	1.357 (0.7258-2.4641)	<.001	0.084	0.274

Values are differences in medians between maternal and fetal erythrocyte lipids based on the nonparametric Wilcoxon matched-pairs signed rank test Spearman rank correlation was used as a non-parametric test to test for association between maternal and fetal relative proportions of lipids, spearman rho (rs) is the correlation coefficient, * P<0.01, ** P<0.001.

Table 5-6 Biomagnification process of relative proportions of fatty acids in RBCs absolute quantities (mg/ml):

	Maternal (n=172)	**Cord (n=172)**	*Wilcoxon*	*Spearman*	
Fatty acid profile (%)	Median (Min-Max)	Median (Min-Max)	*p*	r_s	*p*
14:00	0.013(0.003-0.040)	0.011(0.005-0.029)	<.001	0.027	0.725
16:00	0.369(0.261-0.450)	0.389(0.277-0.472)	<.001	0.007	0.924
18:00	0.182(0.132-0.245)	0.217(0.143-0.277)	<.001	**.146***	**0.025**
20:00	0.011(0.003-0.026)	0.013(0.002-0.031)	1.09	-0.013	0.869
22:00	0.015(0.003-0.042)	0.015(0.003-0.024)	0.839	-0.071	0.355
24:00	0.051(0.027-0.086)	0.063(0.029-0.076)	<.001	0.009	0.909
∑SFA	0.639(0.489-0.797)	0.695(0.528-0.845)	<.001	0.024	0.759
16:1ω7	0.008(0.001-0.029)	0.010(0.003-0.025)	0.025	0.037	0.633
18:1ω9	0.154(0.103-0.196)	0.105(0.038-0.150)	.000	**.139***	**0.041**
18:1ω7	0.013(0.007-0.022)	0.017(0.005-0.031)	<.001	0.049	0.522
20:1ω9	0.010(0.001-0.028)	0.007(0.001-0.022)	<.001	**.128***	**0.048**
22:1ω9	0.010(0.002-0.027)	0.008(0.001-0.025)	.002	-0.12	0.115
24:1ω9	0.046(0.028-0.075)	0.039(0.021-0.084)	<.001	0.005	0.944
∑MUFA	0.241(0.169-0.307)	0.186(0.107-0.256)	.000	0.072	0.172
18:2ω6	0.115(0.065-0.182)	0.036(0.013-0.076)	.000	0.098	0.202
18:3ω6	0.010(0.001-0.031)	0.009(0.002-0.022)	<.001	0.042	0.588
20:2ω6	0.010(0.001-0.023)	0.007(0.001-0.023)	<.001	0.005	0.951
20:3ω6	0.017(0.009-0.037)	0.024(0.008-0.051)	<.001	0.086	0.262
20:4ω6	0.149(0.083-0.197)	0.208(0.112-0.287)	.000	**.147***	**0.027**
22:2ω6	0.010(0.000-0.025)	0.007(0.000-0.022)	<.001	-0.069	0.366
22:4ω6	0.025(0.009-0.043)	0.032(0.013-0.063)	<.001	-0.003	0.967
22:5ω6	0.004(0.001-0.009)	0.011(0.004-0.027)	.000	-0.039	0.613
∑ω6	0.335(0.259-0.460)	0.342(0.223-0.461)	<.001	**.168***	**0.015**
18:3 ω3	0.006(0.001-0.013)	0.003(0.000-0.009)	.000	-0.025	0.744
20:3 ω3	0.004(0.002-0.012)	0.006(0.001-0.024)	.004	0.027	0.727
20:5 ω3	0.014(0.003-0.052)	0.007(0.001-0.025)	<.001	0.052	0.496
22:5 ω3	0.015(0.002-0.038)	0.011(0.001-0.027)	<.001	0.026	0.736
22:6 ω3	0.057(0.015-0.098)	0.056(0.028-0.099)	.547	**.198****	**0.005**
∑ω3	0.105(0.048-0.201)	0.087(0.037-0.146)	<.001	**.231****	**0.001**
AA/LA	1.175(0.485-2.494)	5.849(2.342-14.563)	.000	0.027	0.363
AA/DHA	2.333(1.009-10.505)	3.740(1.317-8.094)	<.001	**.245****	**0.001**
ω6/ω3	3.391(1.396-7.008)	3.975(1.708-11.706)	<.001	**.239****	**0.001**
22:5/22:4ω6	0.145(0.044-1.015)	0.335(0.080-1.568)	.000	-0.098	0.203
ω3 Index (EPA+DHA)	0.076(0.024-0.150)	0.066(0.032-0.120)	.003	**.199****	**0.005**
(AA+DHA)/ MUFA	0.831(0.428-1.270)	1.449(0.779-2.661)	.000	.0608	0.187

Values are differences in medians between maternal and fetal erythrocyte lipids based on the nonparametric Wilcoxon matched pairs signed rank test Spearman rank correlation was used as a non-parametric test to test for association between maternal and fetal relative proportions of lipids, spearman rho (rs) is the correlation coefficient, * P<0.01, ** P<0.001

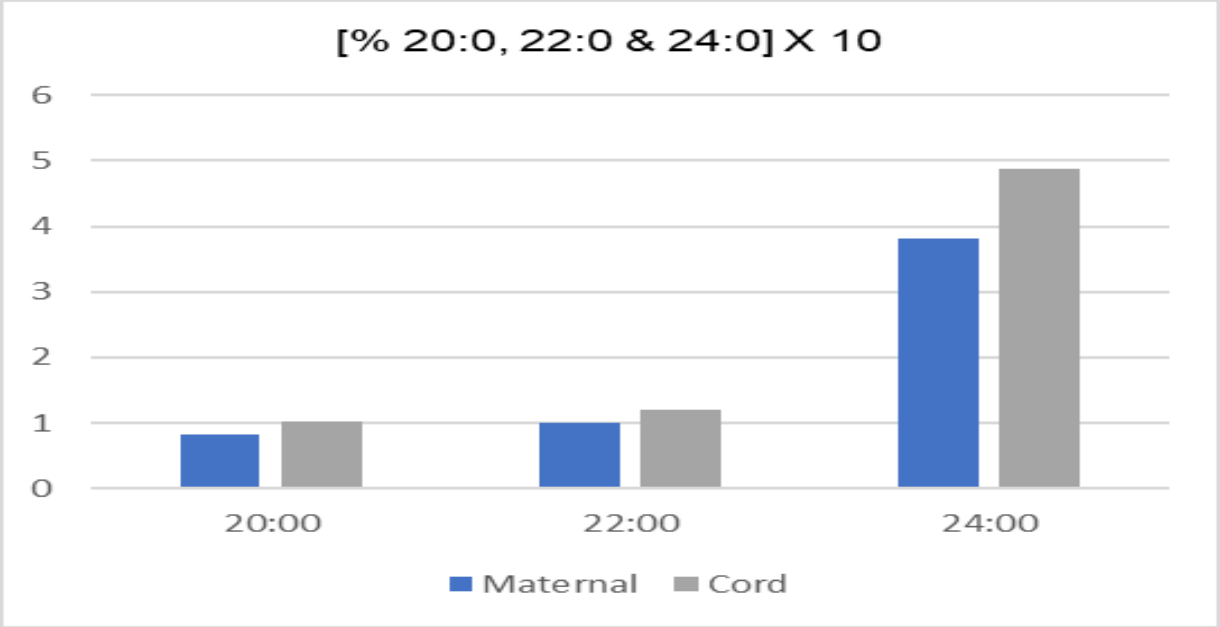

Figure 5-1 Consistent biomagnification of SFAs (C:20. C:20, and C:24) across the placenta in maternal blood at delivery and fetal cord blood.

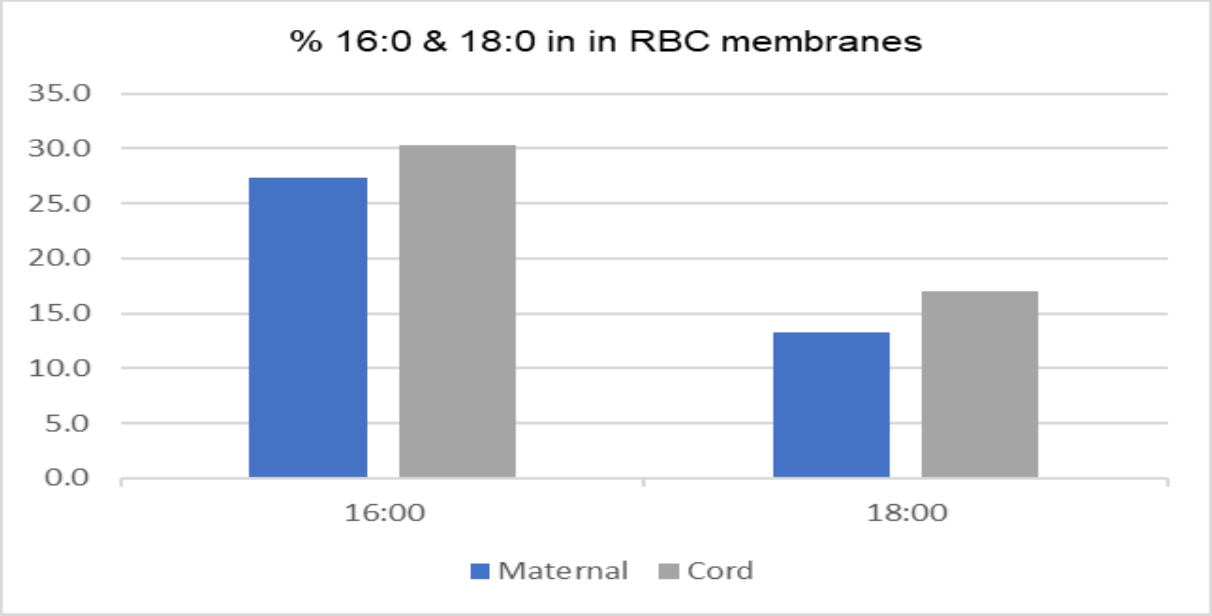

Figure 5-2 Consistent biomagnification of SFAs (C:16 and C:18) across the placenta in maternal blood at delivery and fetal cord blood.

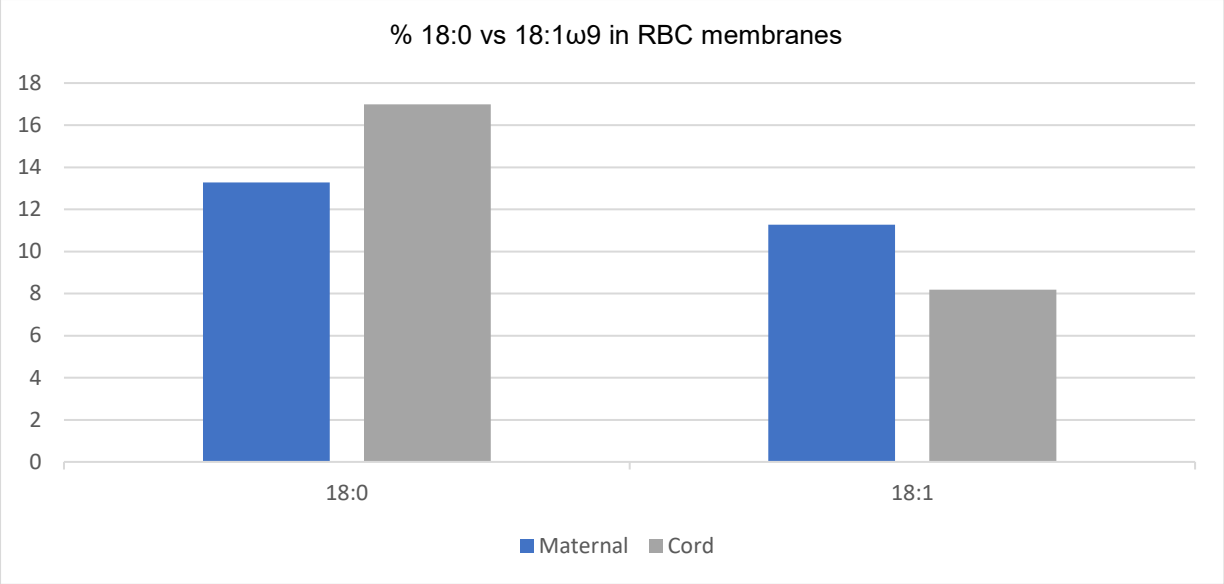

Figure 5-3 consistent biomagnification of Stearic acids (C:18) versus bioreduction process of oleic acid (18:1 ω9) in maternal blood at delivery and fetal cord blood.

5.4.3 Gestational Diabetes

Table 5-7 reports the median percentage of maternal erythrocyte FAs' profile data for the women with GDM and the control group.

Table 5-7 shows that the median rate of total maternal FAs in erythrocytes in the GDM group contained significantly higher levels of palmitic acid (16:00), lignoceric acid (24:00), total saturated fat, oleic acid (18:1n-9), eicosadienoic acid (20:2n-6), adrenic acid (22:4 n-6) Lc omega-6/Lc omega-3 and omega-6/omega-3 ratio (p = <0.001), with regard to the healthy women. Conversely, women with GDM had lower levels of arachidonic acid (20:4 n-6), eicosatrienoic acid (20:3 n-3), docosahexaenoic acid (22:6 n-3) (p = 0.002), total omega-3 (p = 0.001), Lc omega-3, omega-3 index (p = 0.002), AA/LA, 22:5/22:4ω6 and (AA+DHA)/MUFA ratios than the women in the control group. The associations between maternal FAs in absolute quantities (mg/ml) in women with GDM vs the healthy control group are shown in Table 5-8. Women with GDM had significantly lower levels of arachidonic acid (20:4 n-6), eicosatrienoic acid (20:3 n-3), docosapentaenoic acid (22:5 n-3), eicosapentaenoic acid (20:5 n-3), docosahexaenoic acid (22:6 n-3) (p = 0.004), total omega-3 (p = 0.001), Lc omega-3, omega-3 index (p = 0.002), AA/LA, 22:5/22:4ω6 and (AA+DHA)/MUFA ratios, but a higher level of lignoceric acid (24:00), eicosadienoic acid (20:2ω6), Lc omega-6/Lc omega-3 and omega-6/omega-3 ratio (p = <0.001).

Table 5-9 reports the median percentage of the cord erythrocyte FAs' profile data for women with GDM and for controlled group. The median proportion of total cord FAs in erythrocytes in the GDM group was significantly higher in terms of stearic acid (18:00), lignoceric acid (24:00), total saturated fat, and dihomo-γ-linolenic acid (20:3 n-6), but a significantly lower in terms of linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6), docosadiienoic acid (22:2 n-6), adrenic acid (22:4 n-6) and total omega-6 FAs.

Moreover, women in the GDM group had lower cord FAs in erythrocytes with regard to eicosapentaenoic acid (20:5 n-3) ($p = 0.021$), eicosatrienoic acid (20:3 n-3) and (AA+DHA)/MUFA ratio compared to the control group. Table 5-10 reports cord erythrocyte FAs in terms of absolute quantities (mg/ml) for the women with GDM and for the healthy control group. The total cord FAs content in terms of erythrocytes in the GDM group had pointedly elevated levels of stearic acid (18:00), total saturated fat, and dihomo-γ-linolenic acid (20:3 n-6) and 22:5/22:4ω6 ratio, but a significantly lower level of linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6), docosadiienoic acid (22:2 n-6), adrenic acid (22:4 n-6) and total omega-6 FAs. Furthermore, women in the GDM group had lower content of cord FAs in erythrocytes with regard to eicosapentaenoic acid (20:5 n-3), eicosatrienoic acid (20:3 n-3) ($p = 0.022$), and the (AA+DHA)/MUFA ratio.

Table 5-7 Associations between maternal FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without GDM:

Fatty acid (% of total FAs in RBCs)	Control (n=101)	GDM (n=56)	
	Median (Min-Max)	Median (Min-Max)	p
14:00	0.93 (0.21-2.7)	0.945 (0.36-2.89)	0.829
16:00	24.12 (20.1-39.28)	26.55 (19.13-32.67)	**0.008**
18:00	13.17 (10.03-17.5)	13.48 (10.15-16.36)	0.806
20:00	0.78 (0.33-1.88)	0.745 (0.19-1.65)	0.344
22:00	1.82 (0.25-2.96)	1.465 (0.22-2.9)	0.112
24;00	3.62 (2.1-5.94)	4.175 (2.06-6.13)	**<.001**
∑SFA	45.51 (37.64-58.19)	47.6 (38.81-56.92)	**0.015**
16:1ω7	0.64 (0.1-1.99)	0.565 (0.11-1.7)	0.739
18:1ω9	10.49 (6.78-13.56)	11.28 (6.12-14.02)	**0.042**
18:1ω7	0.98 (0.48-1.57)	0.96 (0.52-1.95)	0.79
20:1ω9	0.74 (0.25-1.98)	0.68 (0.1-1.96)	0.802
22:1ω9	0.78 (0.13-2.17)	0.685 (0.17-1.87)	0.395
24:1ω9	3.26 (2.14-5.46)	3.385 (2.15-5.21)	0.829
∑MUFA	17.25 (12.99-21.97)	17.93 (13.01-22.65)	0.058
18:2ω6	6.52 (3.09-12.87)	7.5 (4.63-10.97)	0.058
18:3ω6	0.74 (0.16-2.67)	0.72 (0.11-1.81)	0.647
20:2ω6	0.72 (0.1-1.89)	0.965 (0.11-1.92)	**0.033**
20:3ω6	0.97 (0.26-2.61)	1.08 (0.35-2.8)	0.065
20:4ω6	13.8 (8-17.9)	12.78 (7.4-16.98)	**0.025**
22:2ω6	0.75 (0.12-1.86)	0.705 (0-1.94)	0.69
22:4ω6	1.43 (0.26-3.27)	1.7 (0.67-3.09)	**0.045**
22:5ω6	0.5 (0.16-1.98)	0.45 (0.13-1.25)	0.071
Lc ω6	19.29 (10.19-25.68)	19.02 (13.89-23.75)	0.276
∑ω6	26.53 (16.62-35.6)	26.05 (19.48-32.86)	0.743
18:3 ω3	0.62 (0.13-1.76)	0.55 (0.12-1.28)	0.085
20:3 ω3	0.5 (0.14-2.76)	0.33 (0.1-1.79)	**0.014**
20:5 ω3	1.02 (0.23-4.32)	1.025 (0.01-3.7)	0.063
22:5 ω3	1.6 (0.37-3.38)	1.16 (0.35-3.29)	0.085
22:6 ω3	5.9 (1.29-9.02)	2.98 (1.18-7)	**0.002**
Lc ω3	9.42 (3.14-14.56)	6.285 (3.27-12.89)	**0.001**
∑ω3	9.97 (4.37-15.2)	6.5 (3.87-13.97)	**<.001**
AA/LA	2.04 (0.7-4.32)	1.675 (0.74-3.12)	**0.01**
AA/DHA	2.56 (1.32-10.79)	3.615 (1.29-12.42)	0.103
ω6/ω3	2.65 (1.63-6.99)	3.67 (1.86-7.21)	**<.001**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	3.0088 (1.09-5.74)	**0.002**
22:5/22:4ω6	0.41 (0.06-3.33)	0.2 (0.06-1.2)	**0.003**
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	4.505 (1.87-9.59)	**0.002**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.92 (0.59-1.37)	**<.001**

Table 5-8 Associations between maternal FAs levels in absolute quantities (mg/ml) of RBCs in pregnant women with and without GDM:

	control group (n=101)	**GDM (n=56)**	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.0125 (0.0029-0.0378)	0.01255 (0.005-0.0405)	0.713
16:00	0.3276 (0.2613-0.5499)	0.36835 (0.2614-0.4504)	0.029
18:00	0.1778 (0.1313-0.245)	0.18165 (0.132-0.2248)	0.576
20:00	0.0106 (0.0043-0.0263)	0.01005 (0.0025-0.0231)	0.266
22:00	0.0239 (0.0036-0.0412)	0.0203 (0.0031-0.0377)	0.097
24;00	0.0491 (0.0286-0.0798)	0.05805 (0.0268-0.0858)	**<.001**
∑SFA	0.6075 (0.4893-0.8147)	0.6395 (0.5045-0.7969)	0.092
16:1ω7	0.0084 (0.0013-0.0278)	0.0077 (0.0014-0.0238)	0.627
18:1ω9	0.142 (0.091-0.1899)	0.15005 (0.0857-0.1963)	0.107
18:1ω7	0.0135 (0.0062-0.0218)	0.013 (0.0073-0.0273)	0.617
20:1ω9	0.0101 (0.0033-0.0277)	0.00955 (0.0013-0.0275)	0.753
22:1ω9	0.0102 (0.0018-0.0282)	0.00925 (0.0022-0.0255)	0.331
24:1ω9	0.0448 (0.0279-0.0765)	0.04555 (0.0294-0.0677)	0.956
∑MUFA	0.2341 (0.1818-0.3059)	0.2398 (0.1691-0.3042)	0.219
18:2ω6	0.0884 (0.0433-0.1802)	0.09845 (0.0602-0.1536)	0.105
18:3ω6	0.0099 (0.0021-0.0374)	0.00995 (0.0014-0.0253)	0.515
20:2ω6	0.0095 (0.0013-0.0265)	0.0131 (0.0014-0.025)	**0.046**
20:3ω6	0.0129 (0.0037-0.0365)	0.01455 (0.0046-0.0392)	0.071
20:4ω6	0.1518 (0.088-0.1969)	0.1406 (0.0814-0.1868)	**0.025**
22:2ω6	0.0098 (0.0016-0.0242)	0.00925 (0-0.0252)	0.655
22:4ω6	0.0199 (0.0033-0.0425)	0.0234 (0.0087-0.0433)	0.071
22:5ω6	0.0055 (0.0018-0.0218)	0.0043 (0.0014-0.0138)	0.079
Lc ω6	0.2233 (0.1232-0.359)	0.2227 (0.1607-0.2854)	0.387
∑ω6	0.3203 (0.1994-0.4597)	0.32255 (0.2334-0.4153)	0.862
18:3 ω3	0.0068 (0.0014-0.0194)	0.00605 (0.0013-0.0141)	0.085
20:3 ω3	0.007 (0.0018-0.0386)	0.0046 (0.0014-0.0233)	**0.006**
20:5 ω3	0.0137 (0.003-0.0562)	0.01335 (0.0002-0.0518)	**0.027**
22:5 ω3	0.0176 (0.0041-0.0372)	0.0132 (0.0038-0.0376)	0.162
22:6 ω3	0.078 (0.0167-0.1263)	0.0387 (0.0153-0.098)	**0.004**
Lc ω3	0.1236 (0.0412-0.2846)	0.0779 (0.0443-0.1743)	**<.001**
∑ω3	0.1292 (0.053-0.2076)	0.0822 (0.0475-0.1826)	**<.001**
AA/LA	1.6848 (0.5495-3.3974)	1.37655 (0.5815-2.6393)	**0.01**
AA/DHA	2.0731 (1.114-9.1301)	2.9551 (1.0138-10.5052)	0.084
ω6/ω3	2.4936 (1.5128-6.871)	3.54405 (1.8004-7.0076)	**<.001**
Lc ω6/Lc ω3	1.8462 (1-5.8)	2.6905 (1-5.25)	**0.002**
22:5/22:4ω6	0.3232 (0.0485-2.8205)	0.1614 (0.0443-1.0154)	**0.003**
ω3 Index (EPA+DHA)	0.0958 (0.0289-0.1781)	0.0586 (0.0243-0.1343)	**0.002**
(AA+DHA)/MUFA	0.9281 (0.4388-1.3093)	0.79925 (0.5028-1.19)	**0.001**

Table 5-9 Associations between cord blood FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without GDM:

	Control (n=53)	**GDM (n=40)**	
Fatty acid (% of total FAs in RBCs)	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.87 (0.42-2.3)	0.97 (0.49-2.02)	0.177
16:00	30.34 (22.77-36.68)	31.125 (23-36.9)	0.346
18:00	17.21 (10.79-21.34)	17.985 (12.64-20.71)	**0.035**
20:00	1.08 (0.28-1.99)	0.915 (0.12-1.72)	0.332
22:00	1.16 (0.32-1.83)	1.175 (0.21-1.91)	0.736
24;00	4.4 (2.44-5.88)	4.83 (2.78-5.99)	**0.059**
∑SFA	52.41 (42.03-63.26)	56.13 (44.92-66.05)	**0.033**
16:1ω7	0.76 (0.29-1.98)	0.805 (0.23-2.13)	0.867
18:1ω9	8.58 (4.05-11.71)	8.615 (3.1-10.88)	0.177
18:1ω7	1.3 (0.47-2.38)	1.47 (0.42-2.35)	0.195
20:1ω9	0.62 (0.13-1.45)	0.575 (0.05-1.57)	0.26
22:1ω9	0.59 (0.07-1.92)	0.6 (0.06-1.56)	0.837
24:1ω9	3.08 (2.02-6.54)	3.08 (1.99-4.75)	0.568
∑MUFA	15.3 (9.31-19.96)	15.185 (8.37-18.47)	0.509
18:2ω6	3.2 (1.54-4.73)	2.865 (1.2-4.53)	**0.045**
18:3ω6	0.66 (0.2-1.73)	0.74 (0.16-1.56)	0.392
20:2ω6	0.6 (0.1-1.78)	0.48 (0.12-1.8)	0.109
20:3ω6	1.47 (0.74-3.9)	3.5 (0.6-4.11)	**0.002**
20:4ω6	14.8 (9.62-18.76)	13.785 (9.1-19.01)	**0.028**
22:2ω6	0.65 (0.05-1.26)	0.51 (0.01-1.08)	**0.012**
22:4ω6	2.9 (1.18-4.7)	1.985 (1.03-4.87)	**0.018**
22:5ω6	0.73 (0.32-1.79)	0.825 (0.29-1.72)	0.152
∑ω6	25.47 (16.43-31.48)	24.52 (16.45-33.81)	**0.044**
18:3 ω3	0.3 (0.04-0.56)	0.13 (0.03-1.3)	0.060
20:3 ω3	0.48 (0.08-1.88)	0.33 (0.07-1.58)	**0.021**
20:5 ω3	0.61 (0.21-1.9)	0.505 (0.16-1.63)	**0.047**
22:5 ω3	0.78 (0.08-1.96)	0.655 (0.05-2.05)	0.098
22:6 ω3	4.1 (2.39-7.76)	5.34 (1.67-7.56)	0.186
∑ω3	6.47 (3.94-11.4)	7.115 (2.93-10.5)	0.712
AA/LA	4.83 (3.01-10.2)	5.355 (2.4-13.11)	0.223
AA/DHA	3.35 (1.33-7.21)	3.09 (1.2-6.03)	**0.049**
ω6/ω3	3.93 (1.76-7.86)	3.535 (1.61-7.55)	0.219
22:5/22:4ω6	0.24 (0.07-1.3)	0.39 (0.07-1.43)	0.070
ω3 Index (EPA+DHA)	4.92 (2.61-9.38)	5.595 (2.27-8.98)	0.322
(AA+DHA)/MUFA	1.35 (0.73-2.36)	1.21 (0.66-2.3)	**0.038**

Table 5-10 Associations between cord blood FAs levels in absolute quantities (mg/ml) of RBCs in pregnant women with and without GDM:

	Control group (n=53)	**GDM (n=40)**	
Fatty acid profile (mg/mL)	Median (Min-Max)	Median (Min-Max)	***p***
14:00	0.0111 (0.0054-0.0294)	0.0123 (0.0063-0.0233)	0.24
16:00	0.3884 (0.2915-0.4695)	0.3984 (0.2944-0.4723)	0.348
18:00	0.2203 (0.1381-0.2732)	0.23025 (0.1618-0.2651)	**0.035**
20:00	0.0138 (0.0036-0.0255)	0.0117 (0.0015-0.022)	0.332
22:00	0.0148 (0.0041-0.0234)	0.01505 (0.0027-0.0244)	0.736
24;00	0.0563 (0.0312-0.0752)	0.0618 (0.0356-0.0766)	0.059
∑SFA	0.6708 (0.538-0.8097)	0.7184 (0.575-0.8454)	**0.033**
16:1ω7	0.0097 (0.0037-0.0253)	0.01035 (0.0029-0.0273)	0.917
18:1ω9	0.1098 (0.0518-0.1499)	0.1103 (0.0397-0.1393)	0.178
18:1ω7	0.0166 (0.006-0.0305)	0.0188 (0.0054-0.03)	0.199
20:1ω9	0.0079 (0.0017-0.0186)	0.00735 (0.0006-0.0201)	0.265
22:1ω9	0.0076 (0.0009-0.0246)	0.00765 (0.0008-0.02)	0.834
24:1ω9	0.0394 (0.0259-0.0837)	0.0394 (0.0255-0.0608)	0.568
∑MUFA	0.1958 (0.1192-0.2555)	0.19435 (0.1071-0.2364)	0.509
18:2ω6	0.041 (0.0197-0.0605)	0.03665 (0.0154-0.058)	**0.045**
18:3ω6	0.0084 (0.0026-0.0221)	0.0095 (0.002-0.02)	0.392
20:2ω6	0.0077 (0.0013-0.0228)	0.00615 (0.0015-0.023)	0.107
20:3ω6	0.0188 (0.0095-0.0499)	0.0448 (0.0077-0.0526)	**0.002**
20:4ω6	0.2072 (0.1347-0.2626)	0.193 (0.1274-0.2674)	**0.028**
22:2ω6	0.0083 (0.0006-0.0161)	0.0065 (0.0001-0.0138)	**0.012**
22:4ω6	0.0371 (0.0151-0.0602)	0.02545 (0.0132-0.0623)	**0.018**
22:5ω6	0.0102 (0.0045-0.0251)	0.01155 (0.0041-0.0241)	0.146
∑ω6	0.3455 (0.2234-0.4229)	0.3329 (0.2248-0.4612)	**0.042**
18:3 ω3	0.0042 (0.0006-0.0078)	0.0018 (0.0004-0.0182)	0.056
20:3 ω3	0.0061 (0.001-0.0241)	0.0042 (0.0009-0.0202)	**0.022**
20:5 ω3	0.0078 (0.0027-0.0243)	0.00645 (0.002-0.0209)	**0.047**
22:5 ω3	0.0109 (0.0011-0.0274)	0.00915 (0.0007-0.0287)	0.201
22:6 ω3	0.0525 (0.0306-0.0993)	0.06835 (0.0214-0.0968)	0.186
∑ω3	0.0853 (0.051-0.1461)	0.0921 (0.0513-0.1355)	0.678
AA/LA	5.287 (3.2881-11.1511)	5.8559 (2.6293-14.3422)	0.223
AA/DHA	3.6613 (1.4525-7.8851)	3.3813 (1.3166-6.5958)	0.053
ω6/ω3	4.0494 (1.8441-8.2344)	3.60775 (1.7077-7.2554)	0.185
22:5/22:4ω6	0.2601 (0.0814-1.4187)	0.4158 (0.0666-1.5677)	**0.047**
ω3 Index (EPA+DHA)	0.063 (0.0334-0.1201)	0.07165 (0.0291-0.1149)	0.322
(AA+DHA)/MUFA	1.4384 (0.7836-2.516)	1.2737 (0.711-2.4497)	**0.031**

5.4.4 Preeclampsia

Table 5-11 reports the median percentage of maternal erythrocyte FAs' profile data for women with PE and the healthy control group. As shown in Table 5-11, the median rate of total maternal FAs in erythrocytes in the PE showed significantly higher levels of palmitic acid (16:00), lignoceric acid (24:00), total saturated fat, total omega-6 FAs, Lc omega-6/Lc omega-3 and omega-6/omega-3 ratio (p = <0.006) compared to the control group. Conversely, women with PE had lower levels of docosahexaenoic acid (22:6 n-3) (p = 0.018), total omega-3 (p = 0.017), Lc omega-3, omega-3 index (p = 0.036) than did women in the healthy group.

The associations between maternal FAs in absolute quantities (mg/ml) in women with PE vs the control group are shown in Table 5-12. Women with PE had pointedly low levels of docosahexaenoic acid (22:6n-3) (p = 0.004), total omega-3 (p = 0.022), Lc omega-3 and omega-3 index (p = 0.031), but a higher level of palmitic acid (16:00), lignoceric acid (24:00), total saturated FAs, AA/DHA ratio (p = 0.008), Lc omega-6/Lc omega-3 and omega-6/omega-3 ratio (p = <0.006), compared with the healthy women.

Table 5-13 and Table 5-14 reports the median percentage and absolute quantities (mg/ml) of cord erythrocyte FAs' profile data for women with PE and for healthy women. No differences were observed in either the median percentage or absolute quantities (mg/ml) of cord erythrocyte FAs concentrations between the women with PE and those without.

Table 5-11 Associations between maternal FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without preeclampsia:

Fatty acid (% of total FAs in RBCs)	Control (n=101)	Preeclampsia (n=13)	
	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.93 (0.21-2.7)	0.91 (0.44-2.5)	0.817
16:00	24.12 (20.1-39.28)	27.45 (22.56-32.67)	**0.011**
18:00	13.17 (10.03-17.5)	13.73 (10.22-16.5)	0.772
20:00	0.78 (0.33-1.88)	0.87 (0.19-1.66)	0.541
22:00	1.82 (0.25-2.96)	1.79 (0.99-2.36)	0.799
24;00	3.62 (2.1-5.94)	4.74 (2.29-6.02)	**0.015**
∑SFA	45.51 (37.64-58.19)	48.83 (42.1-53.86)	**0.003**
16:1ω7	0.64 (0.1-1.99)	0.68 (0.33-1.39)	0.42
18:1ω9	10.49 (6.78-13.56)	10.74 (8.62-12.7)	0.748
18:1ω7	0.98 (0.48-1.57)	0.94 (0.68-1.34)	0.728
20:1ω9	0.74 (0.25-1.98)	0.79 (0.39-1.5)	0.982
22:1ω9	0.78 (0.13-2.17)	0.85 (0.43-1.3)	0.782
24:1ω9	3.26 (2.14-5.46)	3.77 (2.64-5.21)	0.322
∑MUFA	17.25 (12.99-21.97)	17.78 (16.01-20.76)	0.405
18:2ω6	6.52 (3.09-12.87)	8.2 (3.91-10.87)	0.354
18:3ω6	0.74 (0.16-2.67)	0.83 (0.39-2.19)	0.356
20:2ω6	0.72 (0.1-1.89)	0.69 (0.35-1.77)	0.838
20:3ω6	0.97 (0.26-2.61)	0.93 (0.56-2.77)	0.876
20:4ω6	13.8 (8-17.9)	14.11 (11.6-16.5)	0.305
22:2ω6	0.75 (0.12-1.86)	0.68 (0.32-1.98)	0.779
22:4ω6	1.43 (0.26-3.27)	1.46 (0.71-3.09)	0.177
22:5ω6	0.5 (0.16-1.98)	0.54 (0.16-1.44)	0.865
Lc ω6	19.29 (10.19-25.68)	20.22 (17.1-24.57)	0.078
∑ω6	26.53 (16.62-35.6)	28.13 (21.55-32.77)	**0.049**
18:3 ω3	0.62 (0.13-1.76)	0.78 (0.43-1.19)	0.577
20:3 ω3	0.5 (0.14-2.76)	0.33 (0.11-1.32)	0.495
20:5 ω3	1.02 (0.23-4.32)	1 (0.34-2.98)	0.229
22:5 ω3	1.6 (0.37-3.38)	1.09 (0.63-3.42)	0.115
22:6 ω3	5.9 (1.29-9.02)	3.04 (1.98-6.4)	**0.018**
Lc ω3	9.42 (3.14-14.56)	6.44 (4.12-10.34)	**0.025**
∑ω3	9.97 (4.37-15.2)	7.17 (4.88-11.13)	**0.017**
AA/LA	2.04 (0.7-4.32)	1.94 (1.19-3.78)	0.738
AA/DHA	2.56 (1.32-10.79)	4.9 (1.87-7.13)	**0.011**
ω6/ω3	2.65 (1.63-6.99)	3.85 (2.23-6.33)	**0.006**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	3.0484 (1.77-5.23)	**0.005**
22:5/22:4ω6	0.41 (0.06-3.33)	0.21 (0.12-0.98)	0.571
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	4.23 (2.32-8.7)	**0.036**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.97 (0.75-1.25)	0.327

Table 5-12 Associations between maternal FAs levels in absolute quantities (mg/ml) of RBCs in pregnant women with and without preeclampsia:

Fatty acid profile (mg/mL)	Control group (n=101)	Preeclampsia (n=13)	
	Median (Min-Max)	Median (Min-Max)	***p***
14:00	0.0125 (0.0029-0.0378)	0.0127 (0.0057-0.035)	0.844
16:00	0.3276 (0.2613-0.5499)	0.3741 (0.2933-0.448)	**0.05**
18:00	0.1778 (0.1313-0.245)	0.1785 (0.1328-0.231)	0.975
20:00	0.0106 (0.0043-0.0263)	0.0122 (0.0025-0.0232)	0.646
22:00	0.0239 (0.0036-0.0412)	0.0232 (0.0139-0.0307)	0.932
24;00	0.0491 (0.0286-0.0798)	0.0616 (0.0321-0.0843)	**0.016**
∑SFA	0.6075 (0.4893-0.8147)	0.6379 (0.5661-0.754)	**0.048**
16:1ω7	0.0084 (0.0013-0.0278)	0.0089 (0.0043-0.0194)	0.435
18:1ω9	0.142 (0.091-0.1899)	0.1477 (0.112-0.1778)	0.908
18:1ω7	0.0135 (0.0062-0.0218)	0.0125 (0.0088-0.0188)	0.562
20:1ω9	0.0101 (0.0033-0.0277)	0.0103 (0.0053-0.0196)	0.932
22:1ω9	0.0102 (0.0018-0.0282)	0.0115 (0.0056-0.0169)	0.862
24:1ω9	0.0448 (0.0279-0.0765)	0.049 (0.0354-0.0677)	0.345
∑MUFA	0.2341 (0.1818-0.3059)	0.234 (0.2081-0.2737)	0.705
18:2ω6	0.0884 (0.0433-0.1802)	0.1084 (0.0548-0.1522)	0.412
18:3ω6	0.0099 (0.0021-0.0374)	0.0116 (0.0052-0.0307)	0.392
20:2ω6	0.0095 (0.0013-0.0265)	0.009 (0.0045-0.0248)	0.897
20:3ω6	0.0129 (0.0037-0.0365)	0.0121 (0.0073-0.0359)	0.873
20:4ω6	0.1518 (0.088-0.1969)	0.1552 (0.1276-0.1815)	0.305
22:2ω6	0.0098 (0.0016-0.0242)	0.0089 (0.0045-0.0258)	0.82
22:4ω6	0.0199 (0.0033-0.0425)	0.0204 (0.0093-0.0433)	0.21
22:5ω6	0.0055 (0.0018-0.0218)	0.0059 (0.0018-0.0158)	0.862
Lc ω6	0.2233 (0.1232-0.3323)	0.2431 (0.2232-0.2987)	0.07
∑ω6	0.3203 (0.1994-0.4597)	0.3361 (0.2534-0.4081)	0.091
18:3 ω3	0.0068 (0.0014-0.0194)	0.0086 (0.0047-0.0131)	0.565
20:3 ω3	0.007 (0.0018-0.0386)	0.0038 (0.0015-0.0172)	0.237
20:5 ω3	0.0137 (0.003-0.0562)	0.013 (0.0044-0.0417)	0.165
22:5 ω3	0.0176 (0.0041-0.0372)	0.0142 (0.0069-0.0376)	0.365
22:6 ω3	0.078 (0.0167-0.1263)	0.0395 (0.0257-0.0868)	**0.019**
Lc ω3	0.1236 (0.0466-0.219)	0.0799 (0.05782-0.1467)	**0.026**
∑ω3	0.1292 (0.053-0.2076)	0.0898 (0.0635-0.1502)	**0.022**
AA/LA	1.6848 (0.5495-3.3974)	1.592 (0.9338-2.9721)	0.848
AA/DHA	2.0731 (1.114-9.1301)	3.8533 (1.47-6.0299)	**0.008**
ω6/ω3	2.4936 (1.5128-6.871)	3.6772 (2.073-6.204)	**0.009**
Lc ω6/Lc ω3	1.8462 (1-5.8)	2.8571 (1.57-4.8)	**0.005**
22:5/22:4ω6	0.3232 (0.0485-2.8205)	0.1627 (0.0985-0.8318)	0.627
ω3 Index (EPA+DHA)	0.0958 (0.0289-0.1781)	0.0582 (0.0302-0.1218)	0.031
(AA+DHA)/MUFA	0.9281 (0.4388-1.3093)	0.8504 (0.6508-1.1206)	0.305

Table 5-13 Associations between cord blood FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without preeclampsia:

Fatty acid (% of total FAs in RBCs)	Control (n=53)	Preeclampsia (n=8)	
	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.87 (0.42-2.3)	0.955 (0.77-1.31)	0.417
16:00	30.34 (22.77-36.68)	26.13 (21.67-35.87)	0.557
18:00	17.21 (10.79-21.34)	16.09 (12.64-20.05)	0.789
20:00	1.08 (0.28-1.99)	0.905 (0.36-1.4)	0.215
22:00	1.16 (0.32-1.83)	1.115 (0.24-1.65)	0.501
24;00	4.4 (2.44-5.88)	4.38 (3.41-5.97)	0.585
∑SFA	52.41 (42.03-63.26)	50.94 (41.24-62.59)	0.669
16:1ω7	0.76 (0.29-1.98)	0.925 (0.33-2.07)	0.305
18:1ω9	8.58 (4.05-11.71)	6.925 (4.54-10.57)	0.521
18:1ω7	1.3 (0.47-2.38)	1.35 (0.76-1.76)	0.856
20:1ω9	0.62 (0.13-1.45)	0.42 (0.26-0.82)	0.132
22:1ω9	0.59 (0.07-1.92)	0.625 (0.28-1.39)	0.685
24:1ω9	3.08 (2.02-6.54)	3.51 (2.47-4.68)	0.399
∑MUFA	15.3 (9.31-19.96)	14.14 (10.88-17.93)	0.677
18:2ω6	3.2 (1.54-4.73)	2.835 (1.05-3.67)	0.685
18:3ω6	0.66 (0.2-1.73)	0.74 (0.2-1.25)	0.42
20:2ω6	0.6 (0.1-1.78)	0.7 (0.15-1.01)	0.89
20:3ω6	1.47 (0.74-3.9)	2.86 (1.04-4)	0.732
20:4ω6	14.8 (9.62-18.76)	16.025 (13.98-17.76)	0.116
22:2ω6	0.65 (0.05-1.26)	0.805 (0.07-1.17)	0.223
22:4ω6	2.9 (1.18-4.7)	3.15 (1.08-4.87)	0.508
22:5ω6	0.73 (0.32-1.79)	0.91 (0.5-1.26)	0.369
∑ω6	25.47 (16.43-31.48)	27.54 (24.43-31.9)	0.14
18:3 ω3	0.3 (0.04-0.56)	0.145 (0.07-0.34)	0.279
20:3 ω3	0.48 (0.08-1.88)	0.58 (0.26-1.13)	0.305
20:5 ω3	0.61 (0.21-1.9)	0.63 (0.07-0.95)	0.631
22:5 ω3	0.78 (0.08-1.96)	0.84 (0.06-1.26)	0.839
22:6 ω3	4.1 (2.39-7.76)	5.065 (2.4-6.72)	0.74
∑ω3 ok	6.47 (3.94-11.4)	7.355 (2.86-9.47)	0.814
AA/LA	4.83 (3.01-10.2)	5.67 (4.46-13.31)	0.276
AA/DHA	3.35 (1.33-7.21)	3.225 (2.28-7.4)	0.94
ω6/ω3	3.93 (1.76-7.86)	3.545 (2.95-11.16)	0.932
22:5/22:4ω6	0.24 (0.07-1.3)	0.23 (0.12-1.02)	0.915
ω3 Index (EPA+DHA)	4.92 (2.61-9.38)	5.7 (2.47-7.45)	0.839
(AA+DHA)/MUFA	1.35 (0.73-2.36)	1.495 (1.12-1.73)	0.2

Table 5-14 Associations between cord blood FAs levels in absolute quantities (mg/ml) of RBCs in pregnant women with and without preeclampsia:

Fatty acid profile (mg/mL)	Control group (n=53)	Preeclampsia (n=8)	
	Median (Min-Max)	Median (Min-Max)	***p***
14:00	0.0111 (0.0054-0.0294)	0.0122 (0.0099-0.0168)	0.417
16:00	0.3884 (0.2915-0.4695)	0.33445 (0.2774-0.4591)	0.557
18:00	0.2203 (0.1381-0.2732)	0.20595 (0.1618-0.2566)	0.789
20:00	0.0138 (0.0036-0.0255)	0.0116 (0.0046-0.0179)	0.215
22:00	0.0148 (0.0041-0.0234)	0.0143 (0.0031-0.0211)	0.501
24;00	0.0563 (0.0312-0.0752)	0.0561 (0.0436-0.0764)	0.548
∑SFA	0.6708 (0.538-0.8097)	0.652 (0.5279-0.8012)	0.669
16:1ω7	0.0097 (0.0037-0.0253)	0.01185 (0.0042-0.0253)	0.305
18:1ω9	0.1098 (0.0518-0.1499)	0.0886 (0.0581-0.1353)	0.521
18:1ω7	0.0166 (0.006-0.0305)	0.01725 (0.0097-0.0225)	0.856
20:1ω9	0.0079 (0.0017-0.0186)	0.00535 (0.0033-0.0105)	0.132
22:1ω9	0.0076 (0.0009-0.0246)	0.008 (0.0036-0.0178)	0.685
24:1ω9	0.0394 (0.0259-0.0837)	0.04495 (0.0316-0.0599)	0.399
∑MUFA	0.1958 (0.1192-0.2555)	0.181 (0.1393-0.2295)	0.677
18:2ω6	0.041 (0.0197-0.0605)	0.0363 (0.0134-0.047)	0.685
18:3ω6	0.0084 (0.0026-0.0221)	0.0095 (0.0026-0.016)	0.42
20:2ω6	0.0077 (0.0013-0.0228)	0.009 (0.0019-0.0129)	0.89
20:3ω6	0.0188 (0.0095-0.0499)	0.0366 (0.0133-0.0512)	0.14
20:4ω6	0.2072 (0.1347-0.2626)	0.22435 (0.1957-0.2486)	0.116
22:2ω6	0.0083 (0.0006-0.0161)	0.0103 (0.0009-0.015)	0.223
22:4ω6	0.0371 (0.0151-0.0602)	0.0403 (0.0138-0.0623)	0.508
22:5ω6	0.0102 (0.0045-0.0251)	0.01275 (0.007-0.0176)	0.352
∑ω6	0.3455 (0.2234-0.4229)	0.37325 (0.3308-0.4304)	0.057
18:3 ω3	0.0042 (0.0006-0.0078)	0.00205 (0.001-0.0048)	0.279
20:3 ω3	0.0061 (0.001-0.0241)	0.00745 (0.0033-0.0145)	0.305
20:5 ω3	0.0078 (0.0027-0.0243)	0.0081 (0.0009-0.0122)	0.631
22:5 ω3	0.0109 (0.0011-0.0274)	0.0118 (0.0008-0.0176)	0.839
22:6 ω3	0.0525 (0.0306-0.0993)	0.0648 (0.0307-0.086)	0.74
∑ω3	0.0853 (0.051-0.1461)	0.09545 (0.0368-0.1231)	0.814
AA/LA	5.287 (3.2881-11.1511)	6.2032 (4.8728-14.5625)	0.276
AA/DHA	3.6613 (1.4525-7.8851)	3.5245 (2.4919-8.0938)	0.949
ω6/ω3	4.0494 (1.8441-8.2344)	3.7024 (3.1145-11.706)	0.915
22:5/22:4ω6	0.2601 (0.0814-1.4187)	0.2547 (0.1267-1.114)	0.932
ω3 Index (EPA+DHA)	0.063(0.0334-0.1201)	0.07295 (0.0316-0.0954)	0.839
(AA+DHA)/MUFA	1.4384 (0.7836-2.516)	1.60285 (1.2172-1.851)	0.207

5.4.5 Spontaneous Preterm birth

Table 5-15 reports the median percentage of maternal erythrocyte FAs' profile data for women with spontaneous preterm births and for healthy control women. As shown in Table 5-15 the median rate of total maternal FAs in erythrocytes in the spontaneous preterm group had significantly higher levels of stearic acid (18:00) (p = <0.018), palmitoleic acid (16:1n-7), (p = <0.009), oleic acid (18:1 n-9) (p = <0.049), cis-vaccenic acid (C18:1n-7), total MUFA, AA/DHA and omega-6/omega-3 ratio (p = <0.027), but lower levels of eicosapentaenoic acid (20:5 n-3), docosahexaenoic acid (22:6 n-3) (p = 0.024), total omega-3 (p = 0.003), omega-3 index (p = 0.023), and (AA+DHA)/MUFA ratio compared to healthy women. The associations between maternal FAs in absolute quantities (mg/ml) in women with spontaneous preterm vs the control group are shown in Table 5-16. Women with spontaneous preterm births had significantly lower levels of docosahexaenoic acid (22:6 n-3) (p = 0.041), total omega-3 (p = 0.005), Lc omega-3, omega-3 index (p = 0.038), and (AA+DHA)/MUFA ratio. Compared to healthy control women, preterm women had significantly lower levels of stearic acid (18:00) (p = <0.016), palmitoleic acid (16:1n-7), (p = <0.008), oleic acid (18:1 n-9) (p = <0.05), cis-vaccenic acid (C18:1n-7), total MUFA, AA/DHA Lc omega-6/Lc omega-3 and omega-6/omega-3 ratio (p = <0.003).

Table 5-17 reports the median percentage of cord erythrocyte FAs' profile data for women with spontaneous preterm and for healthy control women. The median rate of total cord FAs in erythrocytes in the spontaneous preterm group showed significantly higher levels of lignoceric acid (24:00), osbond acid (C22:5n-6) and AA/LA ratio (p = <0.028). Only linoleic acid (18:2 n-6) (p = 0.05) and eicosapentaenoic acid (20:5 n-3) (p = 0.051) were significantly lower in preterm women than in the control group. Table 5-18 reports cord erythrocyte FAs absolute quantities (mg/ml) for women with

spontaneous preterm and healthy control women. The total cord FAs content in erythrocytes in the spontaneous preterm group had significantly higher levels of stearic acid (18:00), linolenic acid (18:3 n-6), osbond acid (C22:5n-6) Lc omega-6/Lc omega-3 ratio and AA/LA ratio ($p = <0.028$). Similarly, linoleic acid (18:2 n-6) ($p = 0.05$) and eicosapentaenoic acid (20:5 n-3) ($p = 0.047$) were only significantly lower in preterm women compared to the control group.

Table 5-15 Associations between maternal FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without spontaneous preterm:

Fatty acid (% of total FAs in RBCs)	Control (n=101)	Spontaneous Preterm (n=10)	
	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.93 (0.21-2.7)	0.985 (0.49-1.75)	0.817
16:00	24.12 (20.1-39.28)	26 (21.28-30.87)	0.221
18:00	13.17 (10.03-17.5)	15.225 (13.23-15.87)	**0.018**
20:00	0.78 (0.33-1.88)	0.755 (0.44-0.91)	0.262
22:00	1.82 (0.25-2.96)	0.87 (0.25-2.5)	0.11
24;00	3.62 (2.1-5.94)	3.625 (2.3-5.03)	0.617
∑SFA	45.51 (37.64-58.19)	47.34 (42.48-54.4)	0.37
16:1ω7	0.64 (0.1-1.99)	1.1 (0.39-1.95)	**0.009**
18:1ω9	10.49 (6.78-13.56)	12.25 (8.46-13.2)	**0.049**
18:1ω7	0.98 (0.48-1.57)	1.37 (0.69-1.67)	**0.017**
20:1ω9	0.74 (0.25-1.98)	0.86 (0.16-1.27)	0.765
22:1ω9	0.78 (0.13-2.17)	1.02 (0.43-1.67)	0.513
24:1ω9	3.26 (2.14-5.46)	3.315 (2.5-4.08)	0.416
∑MUFA	17.25 (12.99-21.97)	19.52 (15.33-21.95)	**0.023**
18:2ω6	6.52 (3.09-12.87)	6.07 (3.1-10.78)	0.934
18:3ω6	0.74 (0.16-2.67)	0.85 (0.43-1.58)	0.785
20:2ω6	0.72 (0.1-1.89)	0.92 (0.36-1.65)	0.151
20:3ω6	0.97 (0.26-2.61)	0.83 (0.7-2.23)	0.632
20:4ω6	13.8 (8-17.9)	14.05 (10.9-14.8)	0.967
22:2ω6	0.75 (0.12-1.86)	0.86 (0.41-1.31)	0.695
22:4ω6	1.43 (0.26-3.27)	1.835 (0.92-2.9)	0.151
22:5ω6	0.5 (0.16-1.98)	0.445 (0.19-1.84)	0.599
Lc ω6	19.29 (10.19-25.68)	20.17 (17.16-21.87)	0.202
∑ω6	26.53 (16.62-35.6)	26.515 (23.1-32.32)	0.797
18:3 ω3	0.62 (0.13-1.76)	0.69 (0.43-0.92)	0.841
20:3 ω3	0.5 (0.14-2.76)	0.34 (0.19-0.75)	0.149
20:5 ω3	1.02 (0.23-4.32)	0.755 (0.34-2.5)	**0.049**
22:5 ω3	1.6 (0.37-3.38)	1.045 (0.77-1.99)	0.085
22:6 ω3	5.9 (1.29-9.02)	2.885 (2.5-6.3)	**0.024**
Lc ω3	9.42 (3.14-14.56)	5.19 (4.47-10.53)	**0.042**
∑ω3	9.97 (4.37-15.2)	5.88 (5.1-11.33)	**0.003**
AA/LA	2.04 (0.7-4.32)	2.04 (1.27-4.26)	0.877
AA/DHA	2.56 (1.32-10.79)	4.79 (2.32-5.76)	**0.026**
ω6/ω3	2.65 (1.63-6.99)	4.62 (2.38-5.17)	**0.004**
Lc ω6/Lc ω3	2.0872 (1.07-6.4)	3.7152 (2.06-4.46)	**0.009**
22:5/22:4ω6	0.41 (0.06-3.33)	0.23 (0.08-0.98)	0.194
ω3 Index (EPA+DHA)	6.96 (2.22-12.72)	3.665 (3.15-8.8)	**0.023**
(AA+DHA)/MUFA	1.07 (0.52-1.52)	0.92 (0.63-1.09)	**0.027**

Table 5-16 Associations between maternal FAs levels in absolute quantities (mg/ml) of RBCs in pregnant women with and without spontaneous preterm:

Fatty acid profile (mg/mL)	Control (n=101)	Spontaneous Preterm (n=10)	
	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.0125 (0.0029-0.0378)	0.0133 (0.0064-0.0245)	0.829
16:00	0.3276 (0.2613-0.5499)	0.3522 (0.2766-0.4322)	0.359
18:00	0.1778 (0.1313-0.245)	0.1993 (0.172-0.2198)	**0.016**
20:00	0.0106 (0.0043-0.0263)	0.0102 (0.0057-0.0126)	0.277
22:00	0.0239 (0.0036-0.0412)	0.0115 (0.0033-0.035)	0.131
24;00	0.0491 (0.0286-0.0798)	0.0495 (0.0322-0.0654)	0.557
∑SFA	0.6075 (0.4893-0.8147)	0.63785 (0.5563-0.7616)	0.291
16:1ω7	0.0084 (0.0013-0.0278)	0.0154 (0.0051-0.0273)	**0.008**
18:1ω9	0.142 (0.091-0.1899)	0.16535 (0.11-0.1848)	**0.051**
18:1ω7	0.0135 (0.0062-0.0218)	0.01845 (0.0097-0.0227)	**0.017**
20:1ω9	0.0101 (0.0033-0.0277)	0.0117 (0.0021-0.0178)	0.738
22:1ω9	0.0102 (0.0018-0.0282)	0.01325 (0.006-0.0227)	0.52
24:1ω9	0.0448 (0.0279-0.0765)	0.04555 (0.035-0.053)	0.468
∑MUFA	0.2341 (0.1818-0.3059)	0.2692 (0.1993-0.3073)	**0.025**
18:2ω6	0.0884 (0.0433-0.1802)	0.0819 (0.0434-0.1502)	0.906
18:3ω6	0.0099 (0.0021-0.0374)	0.0115 (0.0056-0.0221)	0.781
20:2ω6	0.0095 (0.0013-0.0265)	0.0129 (0.0047-0.0215)	0.137
20:3ω6	0.0129 (0.0037-0.0365)	0.01125 (0.0091-0.0313)	0.669
20:4ω6	0.1518 (0.088-0.1969)	0.15455 (0.1199-0.1628)	0.967
22:2ω6	0.0098 (0.0016-0.0242)	0.0116 (0.0053-0.0183)	0.703
22:4ω6	0.0199 (0.0033-0.0425)	0.0248 (0.0129-0.0406)	0.152
22:5ω6	0.0055 (0.0018-0.0218)	0.0049 (0.0021-0.0202)	0.596
Lc ω6	0.223 (0.1232-0.3872)	0.2344 (0.2155-0.2601)	0.167
∑ω6	0.3203 (0.1994-0.4597)	0.32145 (0.2791-0.4072)	0.821
18:3 ω3	0.0068 (0.0014-0.0194)	0.0076 (0.0047-0.0101)	0.841
20:3 ω3	0.007 (0.0018-0.0386)	0.0046 (0.0025-0.0098)	0.166
20:5 ω3	0.0137 (0.003-0.0562)	0.01055 (0.0044-0.035)	0.058
22:5 ω3	0.0176 (0.0041-0.0372)	0.0115 (0.0085-0.0219)	0.085
22:6 ω3	0.078 (0.0167-0.1263)	0.03905 (0.035-0.0882)	**0.041**
Lc ω3	0.123 (0.04702-0.2309)	0.069 (0.0621-0.1438)	0.059
∑ω3	0.1292 (0.053-0.2076)	0.076 (0.0664-0.152)	**0.005**
AA/LA	1.6848 (0.5495-3.3974)	1.6694 (1.0398-3.3456)	0.91
AA/DHA	2.0731 (1.114-9.1301)	3.826 (1.8458-4.5257)	**0.036**
ω6/ω3	2.4936 (1.5128-6.871)	4.3966 (2.1868-4.9725)	**0.003**
Lc ω6/Lc ω3	1.8462 (1-5.8)	3.4286 (1.86-4)	**0.013**
22:5/22:4ω6	0.3232 (0.0485-2.8205)	0.1887 (0.0704-0.7724)	0.184
ω3 Index (EPA+DHA)	0.095(0.0289-0.1781)	0.051(0.0416-0.1232)	**0.038**
(AA+DHA)/MUFA	0.9281 (0.4388-1.3093)	0.7696 (0.5223-0.9275)	**0.02**

Table 5-17 Associations between cord blood FAs levels (FAs % of total FAs in RBCs) of pregnant women with and without spontaneous preterm:

Fatty acid (% of total FAs in RBCs)	Control (n=53)	Spontaneous Preterm (n=13)	
	Median (Min-Max)	Median (Min-Max)	*p*
16:00	30.34 (22.77-36.68)	27 (23.7-35.42)	0.556
18:00	17.21 (10.79-21.34)	18.1 (13.1-20.9)	0.107
20:00	1.08 (0.28-1.99)	1.07 (0.42-1.8)	0.675
22:00	1.16 (0.32-1.83)	1.24 (0.61-2.87)	0.488
24;00	4.4 (2.44-5.88)	5.13 (2.21-5.96)	**0.025**
∑SFA	52.41 (42.03-63.26)	55.07 (46.47-59.14)	0.535
16:1ω7	0.76 (0.29-1.98)	0.75 (0.34-1.89)	0.663
18:1ω9	8.58 (4.05-11.71)	7.6 (2.81-9.2)	**0.035**
18:1ω7	1.3 (0.47-2.38)	1.35 (0.62-1.85)	0.878
20:1ω9	0.62 (0.13-1.45)	0.7 (0.49-1.57)	0.126
22:1ω9	0.59 (0.07-1.92)	0.7 (0.35-2.26)	0.163
24:1ω9	3.08 (2.02-6.54)	3.08 (2.22-4.18)	0.346
∑MUFA	15.3 (9.31-19.96)	14.17 (9.72-16.67)	0.206
18:2ω6	3.2 (1.54-4.73)	2.28 (1.2-4.21)	**0.05**
18:3ω6	0.66 (0.2-1.73)	0.98 (0.32-1.43)	**0.028**
20:2ω6	0.6 (0.1-1.78)	0.7 (0.13-1.16)	0.567
20:3ω6	1.47 (0.74-3.9)	1.38 (0.61-3.88)	0.878
20:4ω6	14.8 (9.62-18.76)	14.98 (9.42-20.5)	0.425
22:2ω6	0.65 (0.05-1.26)	0.58 (0.07-1.74)	0.415
22:4ω6	2.9 (1.18-4.7)	2.08 (0.9-4.67)	0.652
22:5ω6	0.73 (0.32-1.79)	0.92 (0.35-1.6)	**0.05**
∑ω6	25.47 (16.43-31.48)	25.9 (18.19-32.34)	0.815
18:3 ω3	0.3 (0.04-0.56)	0.1 (0.03-1.4)	0.426
20:3 ω3	0.48 (0.08-1.88)	0.54 (0.14-1.22)	0.242
20:5 ω3	0.61 (0.21-1.9)	0.38 (0.2-1.1)	0.051
22:5 ω3	0.78 (0.08-1.96)	0.34 (0.1-1.76)	0.058
22:6 ω3	4.1 (2.39-7.76)	3.5 (1.2-7.56)	0.443
∑ω3	6.47 (3.94-11.4)	6.08 (3.74-8.92)	0.287
AA/LA	4.83 (3.01-10.2)	6.83 (3.75-10.1)	**0.028**
AA/DHA	3.35 (1.33-7.21)	4.02 (1.25-12.48)	0.194
ω6/ω3	3.93 (1.76-7.86)	4.13 (2.16-7.1)	0.325
22:5/22:4ω6	0.24 (0.07-1.3)	0.39 (0.09-1.66)	0.205
ω3 Index (EPA+DHA)	4.92 (2.61-9.38)	4.26 (1.72-7.94)	0.252
(AA+DHA)/MUFA	1.35 (0.73-2.36)	1.46 (0.91-2.46)	0.354

Table 5-18 Associations between cord blood FAs proportions in absolute quantities (mg/ml) of RBCs in pregnant women with and without spontaneous preterm:

Fatty acid profile (mg/mL)	Control (n=53)	Spontaneous Preterm (n=13)	
	Median (Min-Max)	Median (Min-Max)	*p*
14:00	0.0111 (0.0054-0.0294)	0.0116 (0.0072-0.0233)	0.406
16:00	0.3884 (0.2915-0.4695)	0.3456 (0.3034-0.4534)	0.556
18:00	0.2203 (0.1381-0.2732)	0.2317 (0.1677-0.2675)	0.109
20:00	0.0138 (0.0036-0.0255)	0.0137 (0.0054-0.023)	0.675
22:00	0.0148 (0.0041-0.0234)	0.0158 (0.0078-0.0367)	0.488
24;00	0.0563 (0.0312-0.0752)	0.0657 (0.0282-0.0763)	**0.025**
∑SFA	0.6708 (0.538-0.8097)	0.7049 (0.5948-0.7569)	0.535
16:1ω7	0.0097 (0.0037-0.0253)	0.0096 (0.0044-0.0242)	0.663
18:1ω9	0.1098 (0.0518-0.1499)	0.0973 (0.0359-0.1178)	**0.035**
18:1ω7	0.0166 (0.006-0.0305)	0.0172 (0.0079-0.0237)	0.859
20:1ω9	0.0079 (0.0017-0.0186)	0.009 (0.0063-0.0201)	0.128
22:1ω9	0.0076 (0.0009-0.0246)	0.009 (0.0045-0.029)	0.163
24:1ω9	0.0394 (0.0259-0.0837)	0.0394 (0.0284-0.0535)	0.346
∑MUFA	0.1958 (0.1192-0.2555)	0.1814 (0.1244-0.2134)	0.206
18:2ω6	0.041 (0.0197-0.0605)	0.0292 (0.0154-0.0539)	**0.05**
18:3ω6	0.0084 (0.0026-0.0221)	0.0125 (0.0041-0.0183)	**0.03**
20:2ω6	0.0077 (0.0013-0.0228)	0.009 (0.0017-0.0149)	0.567
20:3ω6	0.0188 (0.0095-0.0499)	0.0177 (0.0078-0.0497)	0.878
20:4ω6	0.2072 (0.1347-0.2626)	0.2097 (0.1319-0.287)	0.425
22:2ω6	0.0083 (0.0006-0.0161)	0.0074 (0.0009-0.0223)	0.402
22:4ω6	0.0371 (0.0151-0.0602)	0.0266 (0.0115-0.0598)	0.652
22:5ω6	0.0102 (0.0045-0.0251)	0.0129 (0.0049-0.0224)	**0.05**
∑ω6	0.3455 (0.2234-0.4229)	0.3544 (0.2461-0.4388)	0.729
18:3 ω3	0.0042 (0.0006-0.0078)	0.0014 (0.0004-0.0196)	0.426
20:3 ω3	0.0061 (0.001-0.0241)	0.0069 (0.0018-0.0156)	0.242
20:5 ω3	0.0078 (0.0027-0.0243)	0.0049 (0.0026-0.0141)	**0.047**
22:5 ω3	0.0109 (0.0011-0.0274)	0.0048 (0.0014-0.0247)	0.059
22:6 ω3	0.0525 (0.0306-0.0993)	0.0448 (0.0154-0.0968)	0.443
∑ω3	0.0853 (0.051-0.1461)	0.0791 (0.0493-0.1147)	0.266
AA/LA	5.2871 (3.2881-11.1511)	7.474 (4.0983-11.0469)	**0.028**
AA/DHA	3.6613 (1.4525-7.8851)	4.3969 (1.3628-13.6536)	0.194
ω6/ω3	4.0494 (1.8441-8.2344)	4.4031 (2.2768-7.2769)	0.337
22:5/22:4ω6	0.2601 (0.0814-1.4187)	0.5334 (0.1035-1.4583)	0.12
ω3 Index (EPA+DHA)	0.063 (0.0334-0.1201)	0.0545 (0.022-0.1016)	0.252
(AA+DHA)/MUFA	1.4384 (0.7836-2.516)	1.5656 (0.9774-2.6614)	0.371

Table 5-19 and Table 5-20 summarise the associations between the median percentage of maternal and cord erythrocyte fatty acid profile and neonatal birth outcomes (gestational age (days) and customised birthweight centile). As shown in Table 5-19 the linear regression analyses indicate significant positive associations only between the customised birthweight centile and the median percentage of palmitoleic acid (16:1 n-7) (p = 0.01) and oleic acid (18:1 n-9) (p = 0.01). The total saturated FAs (p = 0.018), total omega-3 (<0.001) and omega-6/omega-3 ratios (p = <0.008) were negatively associated with the customised birthweight centile. In terms of the association between the proportion of FAs levels in cord RBCs, the α-linolenic acid (18:3 n-3) (p = <0.02) and the AA/LA ratio (p = <0.012) were positively associated with the customised birthweight centile. Similar results were observed between the FAs (absolute content) of maternal erythrocyte fatty acids and the customised birthweight centile listed in the appendix 10. As shown in Table 5-20, the linear regression analyses indicate significant negative associations only between gestational age and the median percentage of cis-vaccenic acid (C18:1n-7) (p = 0.028). Furthermore, there were significant positive relationships between gestational age and the median percentage of erythrocyte fatty acid FAs in cord blood with regard to lignoceric acid (24:00) (p = 0.01) gondoic acid (20:1 n-9) (p = 0.012) and AA/DHA ratio (p = <0.001). Similar outcomes were reported between the FAs (absolute content) of cord erythrocyte fatty acid and gestational age listed in the appendix 10.

Table 5-19 Linear regression analysis of the relation between maternal RBCs and fetal FAs composition (% of total FAs) and customised birthweight centile of the Omani pregnant women:

Maternal FA (% RBC)				**Cord FA (% RBC)**			
	β	**95%CI**	***p***		**β**	**95%CI**	***p***
∑SFA	-0.159	(-32.588-96.283)	**0.018**	18:3 ω3	0.171	(3.435-39.744)	**0.02**
16:1ω7	0.183	(-1.848--0.177)	**0.01**	AA/LA	0.185	(0.587-4.696)	**0.012**
18:1ω9	0.334	(2.924-21.033)	**<.001**				
20:3 ω3	0.205	(5.582-29.016)	**0.004**				
-∑ω3	-0.738	(-11.328--3.427)	**<.001**				
ω6/ω3	-0.478	(-17.651--2.694)	**0.008**				
(AA+DHA)/MUFA	0.414	(27.356-81.206)	**<.001**				

Table 5-20 Linear regression analysis of the relation between maternal RBCs and fetal FAs composition (% of total FAs) and gestational age (days) of the Omani pregnant women:

Maternal FA (% RBC)				**Cord FA (% RBC)**			
	β	**95%CI**	**p**		**β**	**95%CI**	**p**
18:1ω7	-0.147	(-15.014- -0.886)	**0.028**	24:00	-0.189	(-5.347-0.702)	**0.011**
				AA/DHA	-0.43	(-5.325-1.488)	**<.001**
				ω6/ω3	0.331	(0.895.605)	**0.007**
				20:1ω9	-0.182	(-13.204-1.635)	**0.012**

5.5 Discussion

This study aims to investigate the transmission of FAs from maternal circulation to fetal circulation across the human placenta through the ‘‘biomagnification’’ process of FAs in maternal and cord erythrocytes. This study also observed the association between the maternal nutritional intake of FAs and the maternal FAs’ profile of erythrocytes and maternal and neonatal outcomes among sample of pregnant Omani women.

5.5.1 Fish and omega 3 FAs intake and pregnancy and neonatal outcomes

The study’s outcome shows a significant relationship between fish and omega-3 FAs intake with GDM and that higher DHA intake is significantly associated with longer gestational age. Furthermore, EPA and DHA intake positively linked with the customised birthweight centile. A "customized birthweight centile" is a measurement of a baby's weight percentile that considers maternal factors such as height, weight, and ethnicity, offering a tailored evaluation of fetal growth in comparison to other babies of similar gestational age and sex (689). Consistent with our findings, earlier studies have suggested that DHA is crucial in promoting optimal pregnancy outcomes, particularly in lowering the prevalence of preterm birth and increasing the gestational age (139,305). A meta-analysis and systematic review indicated that omega-3 supplements during pregnancy lead to more extended gestational periods, reduced preterm delivery likelihood, and a lower incidence of low birth weight (73).

A clinical trial revealed that omega-3 FAs control fasting plasma glucose, reduce inflammation, and minimise oxidative stress in individuals with GDM (315). This study also found a lower rate of newborn hospital admission or hospital stays in the treatment group (315). The results could be due to the statement that omega-3 FAs influence

pregnant women's' metabolic biomarkers and enhance bile acid absorption (319,320). Omega-3 FAs have been found to moderate inflammation by decreasing the adhesion of molecules, leukocyte chemotaxis, leukocyte-endothelial interaction, inflammation-induced cytokines, and T-cell reactivity (321). They reduce inflammation by changing cell membrane FAs and inhibiting NF-κB activation (322).

In 2019, Hibbeln and an expert panel undertook a systematic review to assess the advantages and disadvantages of seafood intake during pregnancy (174). This indicated that there was no evidence of any upper limit for intake that would result in adverse effects on harm neurodevelopment. Instead, they highlighted the advantages of a higher intake of various seafoods to enhance cognitive development and pointed to the beneficial nutrients in fish and seafood that can counterbalance any potential negative impacts from exposure to MeHg (174,175). Fish advisories in the USA are guided by the precautionary principle and are based on epidemiological studies that observed people who ate whale and shark meat with high mercury levels (763).

Furthermore, the multi-cohort Seychelles Child Development Study (SCDS) studied a population over a 24-year period that had a fish intake significantly higher than the recommended global intake (8 meals/week) (764) and higher prenatal exposure to MeHg (>5 ppm determined in hair), like those found in UK and US commercial fish (765). The study found no evidence that exposure to MeHg harmed children's neurodevelopment and suggested that the beneficial role of the fish nutrients exceeds the ingestion of MeHg (764,766–769). They found that LCPUFA status during pregnancy is positively linked to early childhood development, whereas omega-3 and omega-6 FAs may help counteract any adverse effects of MeHg exposure (169,768,769).

5.5.2 FAs Biomagnification

This study has revealed the intricate mechanism of the placenta's active selection of specific FAs through biomagnification while rejecting other FAs through bioreduction. Biomagnification through the placenta is primarily regulated by AA and its allies, DGLA, ADA and omega-6 DPA. Stearic acid, which is saturated FAs, is also bio-magnified, probably to offer the sn-1 position in membrane production (770). In contrast, there is a bio-reduction of MUFA, specifically oleic acid and linoleic acids in omega-6 and all omega-3 precursors of DHA and EPA. Although DHA is biomagnified, the amount transferred from the mother to the fetus is relatively low compared to that of AA. Furthermore, the AA precursor, linoleic acid, is processed reversely, with its erythrocyte level in the fetus being half that of the maternal. The fetus rejects it by bioreduction and then sends it to the mother’s circulation through the umbilical arterial cord (771).

This applies to all DHA precursors, including α-linolenic acid and EPA, which are refused by the fetus, as found previously (110). This outcome could be explained by the fact that the LA restrains the use of AA and DHA, and its transformation into AA and DHA in the developing fetus is restricted because desaturase enzymes are absent in the fetus. As a result, it could be returned to the maternal circulation to priorities AA and DHA for the development of the foetus’s brain (329).

Moreover, this study shows that the proportions of saturated FAs are bio-magnified by the placenta, and the proportions of MUFA, including oleic acid, are bio-reduced. Prenatally, saturated FAs are exchanged for MUFA. The most probable explanation for these results is that the saturated FAs are shifted to be esterified beside the LCUFAs for cell membrane

phosphoglycerides (121). Lipid membranes are essential for cell division and fetal brain growth in the late stage of pregnancy (772).

The equally intriguing negative concentration slope of MUFAs and oleic acid is noteworthy. After childbirth, oleic acid is a mother's milk's most significant higher level FAs (329). This reflects a substantial change from the FAs required during pregnancy, primarily polyunsaturated and saturated FAs needed for cell division. In the postpartum period, MUFA becomes the primary FAs necessary for energy and myelination (773). The placenta stores MUFAs for the mother and increases the SFAs supply for the fetus (121). Unlike other MUFAs, this study found that palmitoleic acid (C16:1w7) does not bioreduce in fetal circulation. Previous studies support this result.

The placental biomagnification of DHA and AA for human fetal passage was first documented by (110). Numerous reports have consistently supported this principle (774–778). During fetal development, the brain takes priority and consumes 70% of the energy dedicated to growth. It was initially assumed that DHA was the central FAs transferred from the mother to the fetus. Surprisingly, as found in the current study, the biomagnification of AA was much higher than that of DHA, suggesting that AA may play a critical part in fetal development (121).

During biomagnification, the levels of DHA in the fetus increase and cross into the liver and form lipoproteins, eventually entering the brain. As a result, the brain contains high levels of DHA (779). In contrast, AA is transferred in large quantities from the mother's bloodstream to the fetus through the placenta and remains highly conserved (780).

Interestingly, the study shows that the percentage of AA is roughly twice that of DHA in the mother and nearly three times as much in the fetus.

Typically, membrane phosphoglycerides contain saturated FAs in SN-1 and polyenoic FAs in SN-2 positions. DHA and AA associate more with SFAs in the SN-1 position, particularly stearic acid (C18:0) (781).

5.5.3 FAs levels in red blood cells and pregnancy and neonatal outcomes

5.5.3.1 Gestational Diabetes

This study shows that women with GDM had an elevated proportion of saturated FAs, omega-6 FAs, and omega-6/omega-3 FAs ratio and lower levels of Omega-3 FAs, omega-3 index and AA compared to the control group. This finding is consistent with earlier observational studies (329,330,782).

Research indicates that there could be variances in the FAs levels between women with GDM and healthy pregnant women. Observational studies have revealed that women with GDM tend to have higher saturated FAs (SFAs) and lower unsaturated FAs (USFAs) than their healthy counterparts (329,330). This shift towards SFAs has been linked with insulin resistance, a key feature of GDM (330). However, studies that have examined subclasses of SFAs yielded different findings. For example, studies involving Chinese women revealed a beneficial association between myristic acid (C14:0) and palmitic acid (C16:0) levels and GDM in comparison to healthy women (331,332).

Another study found opposing associations between odd-chain heptadecanoic acid (C17:0), pentadecanoic acid (C15:0), and even full-chain SFAs, with GDM (333). Similar results have been documented in research into T2D (334) and cardiovascular diseases

(335). Moreover, Chinese studies have confirmed these findings by establishing a correlation between elevated levels of long-chain SFAs and an increased likelihood of developing GDM (336).

Women with GDM had low omega-3 FAs levels, specifically DHA and EPA, but higher levels of LA omega-6 FAs while decreasing beneficial MUFAs such as oleic acid. Research has indicated that MUFAs positively impact insulin sensitivity and glucose metabolism by enhancing cellular insulin signalling and promoting glucose uptake into cells, thus improving overall glycemic control (330).

In women with GDM, 1g of omega-3 FAs supplements daily for six weeks significantly decreased inflammation markers and improved pregnancy outcomes (315). In contrast, some researchers demonstrated that providing pregnant women with omega-3 FAs supplements for six weeks did not significantly impact their fasting plasma glucose levels. Nonetheless, it did reduce HOMA-IR through a reduction in serum insulin levels (318). Conversely, 800mg/d of DHA supplement during the middle stage of pregnancy showed no effect on pre-eclampsia or GDM (323).

Vitamins and omega-3 FAs co-supplements improve pregnancy outcomes in women diagnosed with GDM (324,325). In a six-week clinical trial that included a combination of vitamin E and omega-3, the use of FAs supplements showed promising results in terms of improving metabolic markers for patients with GDM (326). Recently, a meta-analysis of four RCTs to assess the benefits of co-supplementation for women with GDM showed a decrease in oxidative stress and glycaemic index, indicating a positive impact (327). Another meta-analysis on the role of omega-3 FAs supplements on GDM patients

revealed a significant decrease in C-reactive protein, HOMA-IR, and fasting plasma glucose levels (328).

5.5.3.2 Preeclampsia

The current study showed that women with PE had a lower level of DHA, total omega-3 and omega-3 index but a higher omega-6/omega-3 ratio than the healthy women in the control group. This observation agrees with the result of previous research. Studies have revealed that pregnant women with PE have low Omega-3 FAs (337,338). Although numerous RCTs have supported the view that omega-3 FAs can lower the PE rate during pregnancy, however, the outcomes have been inconsistent (296,323). An observational study showed a correlation linking omega-3 FAs and reducing the risk of PE in pregnant women. The study found that for every 1% rise in omega-3 FAs levels, there was a 24% decrease in the risk of PE (339). Furthermore, a recent meta-analysis (341) indicated that the researchers evaluated the efficacy of omega-3 FAs supplements in preventing PE and found a slightly positive impact on reducing the incidence of PE and pregnancy-induced hypertension (RR, 0.98; 95% CI, 0.90–1.07; p = 0.652; I2 = 0%).

In contrast, other studies reported different outcomes. According to the available evidence, no indication that taking omega-3 FAs supplements can prevent gestational hypertension during pregnancy (141). For example, (294), in a limited trial involving 328 pregnant women, found no significant impact on the likelihood of developing PE. Furthermore, a meta-analysis of 6 RCTs reviewing the effect of omega-3 FAs supplements in about 4,130 pregnant women failed to show improvements in PE (340). A recent meta-analysis (341) reported a weak positive impact of omega-3 FAs supplementation on lowering the risk of PE.

5.5.3.3 Spontaneous preterm birth

Women in the spontaneous preterm birth group had significantly elevated omega-6/omega-3 ratio levels. The current finding also shows that DHA, EPA, total omega-3 FAs, and omega-3 index were lower among spontaneous preterm women than healthy women in the control group. Three RCTs have assessed the effectiveness of omega-3 FAs supplements in decreasing the risk of LBW and preterm birth (139,285,306) and have revealed that taking DHA supplements in the range of 800 to 600 mg per day is highly advantageous for several reasons. DHA supplements during pregnancy can benefit childbirth weight, which could result in higher levels of DHA being observed in cord blood samples collected at birth (302). However, despite having the same design, other trials have failed to demonstrate a positive effect of a 400 mg/day DHA supplement in preventing LBW and preterm birth (307). Furthermore, omega-3 FAs use does not improve recurrent preterm labour during pregnancy, according to two meta-analyses (302,304). DHA plus EPA or pure DHA supplementation positively impacts slightly the increase in gestational age and birth weight but does not prevent preterm birth (302).

Omega-3 FAs supplementation can decrease the preterm birth rate by 11% and early preterm birth by 42%. This was found in a 2018 Cochrane Review of nine RCTs involving over 5,000 pregnant women (308). Recently, two extensive clinical trials aimed to evaluate the effectiveness of omega-3 FAs supplements in preventing early preterm birth. A study in Australia involving over 5,000 pregnant women reported no significant improvement in premature birth, even with 100 mg/d of EPA and 800 mg/d of DHA omega-3 FAs supplements (309). After a reanalysis of the study data, it has been concluded that a particular group of participants, specifically those with low levels of

omega-3 FAs at the start, can benefit significantly from the intervention (310). A recent trial in the United States involving over 1000 pregnant women aimed to test the effects of omega-3 supplements on early preterm birth. The trial's findings were replicated, showing consistent results (311); the women participating were assigned to take either 200 mg or 800 mg supplements at random. The study revealed that pregnant women with a low initial DHA level and taking the higher dose experienced a reduced early preterm birth rate.

In this study, women in spontaneous preterm had notably elevated levels of some MUFAs, particularly oleic acid. The findings of Ogundipe et al, indicated a correlation between higher levels of oleic acid or MUFA in the red blood cells of mothers at 12 weeks of pregnancy and a higher risk of premature birth and smaller size (329). That could explain this outcome in preparation for physiological events such as pregnancy; nature stores fat reserves that can cover up to 33% of the lactation requirements during the three months (161).

The relationship between oleic acid and MUFA may significantly impact metabolism, indicating a potential lack of essential FAs in the diet (121). In terms of an essential FAs deficiency, the body attempts to maintain unsaturation in membrane lipids by substituting them with LC unsaturated derivative and oleic acid (329).

5.5.4 Strengths and Limitations

The current study presents various strengths and limitations that need to be considered. One significant advantage of this study is that it is the first to include a substantial group of participants (302 pregnant women) in analysing the levels of erythrocyte FAs intake

among pregnant women in Oman. Additionally, it investigates the correlation between FAs intake and levels with both pregnancy and neonatal outcomes. The second strength of the current study is its cohort design. Additionally, measuring RBCs FAs in early pregnancy provides valuable insights, mainly since the nutritional status assessment by FFQ was determined at 8-12 weeks of pregnancy. Furthermore, relevant descriptive factors were included in the study. Again, FAs levels were determined by the FAs percentage in terms of total FAs and absolute quantities. The analyses of both methods did not affect the results as far as FAs classes are concerned. This may be considered a strength of the study. However, this study is subject to certain limitations. First, the assessment of omega-3 FAs intake is based on FFQs. As with other dietary assessment methods, these questionnaires allow possible incorrect recall and false expectations regarding food portion dietary intake sizes. However, this was avoided by using food model samples to enable the participants to estimate the food portion sizes. In addition, the omega-3 FAs FFQs were validated by the erythrocyte FAs levels. Second, all participants in the study had a similar possibility of being misclassified in terms of their result status and exposure status. This might lead to bias in the power of the link between outcome and exposure. Due to its observational nature, unassessed outstanding factors may confound the work. For example, fish intake might function as a proxy for a healthy lifestyle. Therefore, potential confounders with seafood intake or depression were recorded and used as adjustment variables in the data analysis.

A potential drawback of this study is that convenience sampling may restrict the generalizability of the findings to pregnant women in the broader population. Additionally, we could not consider other variables or potential factors, such as genetic variations,

which could have impacted the erythrocyte profiles of the FAs. We also could not determine if differences in maternal FAs profiles affected the size of the newborn's body due to dietary discrepancies. Further research is warranted to fully understand how omega-3 FAs impact gestational age or other pregnancy outcomes. This research should examine genetic factors and other potential mechanisms.

5.6 Conclusion

This is the first study to investigate how the fish and FAs intake among sample of pregnant Omani women impact their pregnancy outcomes. These findings support previous research indicating maternal omega-3 FAs positively affect important perinatal health outcomes. The prospective cohort study’s outcome shows a significant relationship linking fish and omega-3 FAs intake with GDM, PE, and preterm and customised birthweight centiles. Recent research on the biomagnification of saturated FAs and MUFA during delivery has enhanced our knowledge of lipidomics and developmental physiology. AA and DHA play a crucial role in the reproductive process by prioritising different FAs for various functions and periods. For example, AA is essential for cardiovascular health, the immune system, and placental development (747,779,783), while DHA is crucial for brain growth.

Clinical trials on the influence of omega-3 FAs supplements on pregnancy outcomes could be an essential step towards cementing the observed association. Such a study would clarify the possible benefit of omega-3 FAs supplements with regard to both mother and child and provide the necessary evidence for a significant public health initiative. Fish and seafood intake and omega-3 fatty supplementation are highly recommended for women during pregnancy to ensure the mother's and fetus's well-being.

Chapter 6: Vitamin D, Vitamin B12 and Folate and prenatal depression and anxiety symptoms

6.1 Introduction

Folate and vitamin B12 are essential water-soluble vitamins that have a crucial part in the one-carbon metabolism in diets as common forms of glutamate chains (468). They are essential in regular embryogenesis and work as cofactors in converting homocysteine to methionine, a process which is required to synthesise phospholipids and neurotransmitters (470). Vitamin B complex vitamins are essential in fetal and placental development, differentiation, cellular growth and biosynthetic processes (247). They are also critical for creating purine and synthesising DNA, RNA and thymidine nucleotides protein and lipids in the cellular cytoplasm (470). Consequently, folate and vitamin B-12 deficiency can damage cell division and methylation activity (471).

During pregnancy, the fetus receives its vitamin D requirements via the mother's umbilical cord. It provides evidence that maternal vitamin D level is highly linked with the vitamin D level of cord blood (784). Pregnant women are at a higher risk of vitamin D deficiency than non-pregnant women (785).

This can be attributed to several factors, such as the increased needs of the fetus. Exceptionally, women with dark skin or wearing all-covering clothes most of the time, living in northern latitudes, with low levels of outdoor activities, might be susceptible to a Vitamin D deficiency. In addition, having unhealthy dietary patterns can lead to reduced vitamin D intake (786–788). This may be a causal factor in the progress of pre-and post-natal depression (785).

Biologically possible mechanisms of an association between folate and vitamin B12 deficiency and mood disorders, including depression and anxiety, have been proposed, focusing on their role in methionine creation (473,480). The folate-methylation cycle needs vitamins D and B12 as these are essential for the production of, for example, DNA, red blood cells, membrane phospholipids and neurotransmitters (473). Low vitamin B12 and folate status damages methylation and induces hyperhomocysteinemia (481). Both reduce the main neurological molecules and the possible neurotoxic effects of high levels of homocysteine, which may raise the risk of psychological illnesses (468). Folate and vitamin B12 deficiency also cause other neurological problems such as paresthesia, neural tube defects, myelopathy and neuropathy (482). Observational studies have shown a clear link between lower nutritional intake of folate and vitamin B12 and increased depression symptoms (483,484). Moreover, low vitamin B12 levels in older people are associated with a greater number of depression symptoms (474,475), while low folate levels were linked with depression in adolescents and in the adult age group (484,485).

Vitamin D deficiency has received significant attention for its role in preventing healthy bones and its importance in reducing the risk of several health disorders, such as cardiac disease, T2D, infectious and autoimmune disorders, and many cancers (363). Low vitamin D levels may increase the onset of depression symptoms among the general population (364) and cause prenatal depression (365). In addition, vitamin B12 or folate deficiency have been linked with anaemia, dementia, and cognitive dysfunction (472), nutrition malabsorption (468), hyperhomocysteinemia linked to atherosclerotic vascular

disease (473), diarrhoea or constipation, shortness of breath, loss of appetite, fatigue, pale skin, depressed mood and change in mental status (474,475).

Although environmental, psychological, and biological concepts of mental disorders, including depression, have been critically reviewed, the causal mechanisms of depression remain unclear, and various pathophysiologies may be involved (789). Recent studies in the general population indicate significant roles for vitamin B12, folate and vitamin D deficiency with regard to depressive symptoms in adults (475,483,488,790). Low levels of vitamin B12 and folate are linked with maternal depression (247). A deficit of the levels of vitamin B12 and folate in plasma in pregnant women was found to be correlated with prenatal depression (496,497). However, others did not find a link between a low dietary intake of vitamin B12 and folate (481) or folate status in erythrocytes leading to antenatal depression (498) and postpartum depression (478,481,496,498)

Several research has indicated the association between micronutrient deficiency and prenatal depression (277,494) and postpartum depression (401,495). Furthermore, numerous researchers have discussed the association between low vitamin D and prenatal depression (247,791,792) and vitamin B12 and folate levels among pregnant women (496,497). However, these findings have arrived at inconsistent results (398).

As human fetuses develop, they undergo adaptations to cope with poor maternal nutrition. This results in permanent alterations to their metabolism and physiology. These “programmed” changes may cause various diseases that develop later in life, such as HTN, T2DM, CHD and stroke (793). Furthermore, several pieces of evidence indicate a association between vitamin D concentration during pregnancy and LBW (412,413). However, other findings have not replicated this correlation (414–416). Vitamin D

deficiency among pregnant women has been linked with macrosomia (419,420), small gestational age (411,412,425,426), preterm birth (422,423) and GDM (794,795). These pregnancy outcomes are significant, as they are linked with a high risk of disease in later life (411). For example, GDM risks poor mental health and cognitive and learning disabilities in the newborn; moreover, 25% of people with T2DM experience clinically significant depression (678).

Several studies (434–436) and meta-analyses included 21 observational studies (437), indicate that low levels of vitamin D (<25 nmol/L) among pregnant women is linked with a high risk of GDM. However, many studies are needed to show such an association (428,441–443).

It is suggested that vitamin D plays a significant part in regulatory fetal–placental immune responses during pregnancy. However, the pathophysiology of PE due to low maternal vitamin D is unclear (451). Vitamin D controls various cardiovascular reactions and immunomodulatory pathways. Therefore, low vitamin D levels have been assumed to be linked to PE (452,453). Several observational findings (454–457) and meta-analyses (458,459), indicate a relationship between low levels of vitamin D among pregnant women and high rates of PE. However, other studies have not replicated these findings this (421,462,463).

6.2 Aim

This chapter aims to evaluation the prevalence of vitamin D, vitamin B12 and folate deficiency among sample of pregnant Omani women and evaluate the association

between vitamin levels with depressive and anxiety symptoms and maternal and neonatal outcomes among Omani pregnant women.

6.3 Methods

The detailed methods are described in detail in Chapter 2.

6.3.1 Vitamin D analysis

Blood samples (5 ml) were collected from sample of pregnant Omani women in a separating tube and sent to Al-Buraimi Hospital Laboratory for further analysis. A maternal venous blood sample was collected at study enrolment at 8–12 weeks (n=298). Maternal 25(OH)D3 levels were assessed on the same day. A serum total (vitamin D3) was evaluated using the Cobas-e 411 immunoanalyser with a reasonable electrochemiluminescence (ECL) protein binding assay and various reagents acquired from Roche Diagnostics. The calibration ranged from 3 to 70 ng/ml (617). The coefficients of variability for the intra and inter-assay tests were 2.7–5.1% and 1.5–4.6%, respectively.

The vitamin D analysis was done in the Al-Buraimi Hospital laboratory. Vitamin D status was classified as follows: (a) normal range: serum (vitamin D3) levels (> 50 nmol/L), (b) inadequate range: serum (vitamin D3) levels between 31 and 50 nmol/L, and (c) deficient range: serum (vitamin D3) levels (< 30 nmol/L).

6.3.2 Folate and vitamin B12 analysis

To measure the vitamin B12 and folate status, blood samples (5ml) were collected from the Omani women during the 8th–12th week of pregnancy. Blood samples were collected using regular, red-topped vacutainers with no anticoagulant. Within 4 hours of collection, the blood samples were subjected to a centrifuge for around 10 minutes at 4°C. They

were then stored at -20°C and defrosted immediatelyprior to analysis. Serum vitamin B12 and folate levels were tested at the Al-Buraimi Hospital laboratory was evaluated using Roche reagents electrochemiluminescence in a mechanical Cobas Team 411 (Roche). The direct sequence of the assay for folates is 1.6 to 20.0 ng/mL, CVs of 04% at 3.8 ng/mL and 3.1% at 17.6 ng/mL. The assay sequence for vitamin B12 is 84 to 2000 pg/mL, with CVs of 7% at 246 pg/ mL and 5% at 890 pg/mL. Both plasma vitamin B12 and folate measurements were conducted in line with international standards recognised as the certified method agreed upon by the Ministry of Health in Oman for such blood tests. Plasma folate concentrations below 10 nmol/L indicate folate deficiency, while vitamin B12 deficiency is defined as below 150 pmol/L.

6.3.3 Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 21, developed by IBM Corp., Chicago, Illinois, USA. Descriptive statistics were used to describe pregnant women's socioeconomic, demographic, and psychological data. The results were expressed as means and standard deviations (SD) for continuous variables, while categorical variables were presented as frequencies and percentages.

The relationship between vitamin D, vitamin B12 and folate and the level of anxiety and depression symptoms and pregnancy outcomes were matched. For continuous variables, the Mann-Whitney U test was employed. While for categorical variables, the Chi-squared test was used,

To adjust for potentially confounding influences, additional analyses was conducted using multiple regression statistics tests for variables that showed substantial links with

depression and anxiety symptoms at the p ≤0.05 level. The enter method followed by a remove regression method was used in the analysis. All the variables are entered, and then we try to eliminate variables with less contribution to the total variance. The depression and anxiety symptoms score, folate, vitamin B12 and D variables were entered in the first step; the second step involved introducing the sociodemographic variables, and finally, the health and lifestyle variables. The operative confounding of depression and anxiety and folate, vitamin B12 and D were assessed using a sequence of regression statistics tests in which the uncontrolled regression coefficient was linked with that found after the outline of a potential confounder. The detailed statistical analysis was described in detail in Chapter 2.

6.4 Results

The details of the sociodemographic, clinical characteristics, depression and anxiety symptoms and pregnancy and newborn outcomes of the pregnant women who completed the three assessment points in the study are provided in Chapters 1 and 2.

6.4.1 Vitamin D, vitamin B12 and folate levels

The median (interquartile range) with regard to the serum folate, vitamin B12, and vitamin D at 8 – 12 and 24 – 28 weeks of pregnancy and childbirth are shown in Table 6-1. The median concentrations of the serum vitamin D, B12 and folate at 8 – 12 weeks of pregnancy (N = 302) were estimated to be 68.9. nmol/L, 203.8 pmol/L and 44.4 nmol/L, respectively. The median concentrations of serum vitamin D, vitamin B12 and folate at 24 – 28 week of pregnancy (N = 272) were estimated to be 69.3 nmol/L, 202.7 pmol/L, and 44.3 nmol/L, respectively. The median concentrations of serum vitamin D, B12 and folate at childbirth (N = 242) were 68.3 nmol/L, 202.7 pmol/L, and 44.6 nmol/L, respectively. The

study shows that vitamin D and B12 deficiency incidence was 19.9% and 17%, respectively.

Table 6-2 shows the relationships between the participants' demographic and gestational characteristics (confounding factors) with the vitamin levels among the pregnant women. The folate median levels among the pregnant women were positively associated with educational ranking ($p < 0.0001$), occupational status ($p = 0.04$), type of accommodation ($p = 0.03$) and whether or not the pregnancy was planned ($p = 0.04$) as the folate medians were higher among pregnant women with high education levels (degree or above) ($p < 0.0001$), compared to women with primary education level. The folate medians were elevated among employed women (median = 45.40 vs median = 43.45, $p = 0.04$) compared with unemployed women, and in the case of women who live in their own house (homeowners) (median = 45.40 vs median = 42.61, $p = 0.03$) compared with women who live in rented accommodation. Pregnant women who had planned for their pregnancy had higher folate median levels than women who had not prepared in this way (median = 45.40 vs median = 42.19, $p < 0.05$).

Furthermore, the folate levels among the pregnant women were associated with monthly household income, as the median folate level increased with monthly income. The folate medians were higher among women with higher monthly income (above 1000 Omani Rial median = 45.40 vs median = 41.68, $p < 0.0001$) than women with the lowest monthly income. However, this relationship was not statistically significant ($p = 0.04$). None of the other confounding factors were associated with folate median levels.

Regarding vitamin B12, significant associations were only found with regard to the BMI class at pregnancy registration ($p = 0.01$). The folate medians were higher among women

in the healthy weight range (median = 220.69) than women in the obese group (median =195.5). Otherwise, no other confounding factors were associated with vitamin D and B12 medians levels.

Table 6-1 Vitamins levels of the Omani pregnant women at the three assessments points of the study:

Vitamins	at 8 – 12 weeks of pregnancy N = 302		at 8 – 12 weeks of pregnancy N = 272		At birth outcome N = 242	
	Median	Interquartile Range	Median	Interquartile Range	Median	Interquartile Range
Vitamin D	68.9	(52.9-68.9)	69.3	(53.1-82.8)	68.327	29.61
Folate	44.4	(35.9-44.4)	44.3	(35.7-45.4)	44.69	10
Vitamin B12	203.8	(153.1-264.1)	202.7	(151.1-261.8)	202.75	108.78

Table 6-2 Association between the vitamins level of participants and the Sociodemographic and gestational characteristics of the Omani pregnant women in the study (N=302):

Variables	Vitamins					
	Vitamin D		Folate		Vitamin B12	
	Median	*p*	Median	*p*	Median	*p*
Age (Y)		.75		.25		.54
< 20	69.45		40.82		192.1	
20 – 30	69.83		43.17		201.2	
30 – 40	68.34		45.11		212.8	
> 40	64.04		45.4		191.6	
Educational level		.09		**.001**		.95
Basic Education	63.65		37.94		213.6	
Secondary Education	71.09		43.83		202.3	
Degree or above	66.76		45.40		202.9	
Occupational Status		.13		**.04**		.79
Employed	65.51		45.4		202.3	
Unemployed	69.80		43.45		206.3	
Annual Household Income		.33		**.007**		.25
Less than 500 OR	70.46		41.68		195.8	
500 OR – 1000 OR	67.82		45.03		213.9	
Above 1000	63.61		45.4		196.7	
Housing tenure		.95		**.03**		.66
Rent house	70.90		42.61		203.15	

Homeowner	66.76		45.5		209.9	
Live with husband or wife family	69.69		43.25		199.1	
Duration of Marriage		.53		.13		.15
Less than 2 years	63.99		45.4		194.7	
Less than 5 years	68.67		42.72		186.2	
Less than 10 years	70.09		43.56		220.16	
above than 10 years	67.82		45.4		203.95	
Planned pregnancy		.304		**.005**		.12
Yes	69.52		45.4		210.9	
No	68.67		42.19		198.2	
Physical activity		.71		.71		.86
No	69.65		44.65		213.7	
30 minutes for at least 2/ week	69.62		44.20		198.75	
30 minutes for at least 3/week	66.93		45.4		192.75	
30 minutes for at least 5/week	67.55		43.06		206.4	
History of Depression		.15		.79		.27
Yes	72.09		44.08		183	
No	68.65		44.39		208.1	
Family History of Depression		.77		.27		.98
Yes	71.17		45.4		197.05	
No	68.67		43.92		203.75	
Parity		.57		.20		.21
Nulliparous	65.23		45.4		213	
Primiparous	69.34		44.59		179.65	
Multiparous	69.44		43.25		212.34	
Obesity BMI		.76		.31		**.01**
Underweight	69.83		40.63		219.4	
Normal	69.24		44.44		220.69	
Overweight	69.89		44.99		197.7	
Obesity	67.61		44.64		195.5	
Obstetric complication Previous Pregnancy		.59		.42		.64
Miscarriage	69.03		45.4		216.35	
Preeclampsia (PE)	57.53		40.22		177.75	
Preterm	65.51		45.4		208.4	
Mode of Delivery in previous pregnancies		.51		.36		.29
Normal Delivery (SVD)	68.18		43.86		213.14	
Caesarean section (CS)	70.45		43.53		189.20	

6.4.2 Vitamin D, vitamin B12 and folate levels with depression and anxiety symptoms

Table and Table 6-4 show the association between vitamins level of pregnant women at enrolment (8-12 weeks of pregnancy) and depression and anxiety symptoms at weeks 8-12 and weeks 24-28 of pregnancy. There were no significant differences between vitamin B12 (p = 0.24), vitamin D (p = 0.80), and folate status (p = 0.73) and depressive symptoms among pregnant women at 8-12 weeks of pregnancy and vitamin D (p = 0.53), vitamin B12 (p = 0.51), and folate status (p = 0.97) with depressive symptoms among pregnant women at 24-28 week of pregnancy. There were no significant differences between vitamin D (p = 0.46), vitamin B12 (p = 0.16), and folate status (p = 0.36) and anxiety symptoms among pregnant women at 8-12 weeks of pregnancy, and vitamin D (p = 0.52), vitamin B12 (p = 0.18), and folate status (p = 0.29) with anxiety symptoms among study sample at 24 - 28 weeks of pregnancy.

Table 6-5 and Table 6-6 describe the adjusted and unadjusted logistic regression analyses for reports of the vitamins level results for pregnant women with and without antenatal depression and anxiety symptoms and the odds ratio (OR), confidence intervals and P values at 8 – 12 weeks and 24 – 24 weeks of pregnancy. An unadjusted logistic regression of depression symptoms on vitamins level found no association among the study sample at 8 – 12 weeks of pregnancy, vitamin D (OR= 1.001(0.99-1.013), vitamin B12 (OR=0.999 (0.996-1.001) and folate level (OR=0.995 (0.96-1.031). The same also was true when the association was assessed at 24 – 24 weeks of pregnancy in terms of vitamin D (OR= 1.001(.988-1.015), vitamin B12 (OR=.998(.995-1.002) and folate level (OR=1.003(.962-1.047). This result was replicated even after adjusting for potential

confounders. The unadjusted and adjusted logistic regression analyses show no association between all vitamin levels and anxiety symptoms during pregnancy at 8 – 12 weeks and 24 – 24 weeks.

Table 6-3 Association between vitamin level of participants with depression symptoms at week 8-12 and week 24-28 of pregnancy:

			at weeks 8-12 of pregnancy					at weeks 24-28 of pregnancy				
Variables			**Non-depressive** (EPDS≤12) (n=212) (70.2%)		**Depressive** (EPDS) ≥ 13 (n=90) (29.8%)			**Non-depressive** (EPDS≤12) (n=189) (72.5%)		**Depressive** (EPDS) ≥ 13 (n=83) (27.5%)		
	Median	***Interquartile range***	***Median***	***Interquartile range***	***Median***	***Interquartile range***	***p***	***Median***	***Interquartile range***	***Median***	***Interquartile range***	***p***
Vitamin D	68.9	52.9-68.9	68.86	53.6-82.4	68.73	50.9-85.2	.80	69.69	27.72	68.67	36.85	.53
Folate	44.4	35.9-44.4	44.39	36.43-45.4	44.26	34.7-45.4	.73	43.94	10	45.4	10	.97
Vitamin B12	203.8	153.1-264.1	208.85	154-267.9	192.5	146.9-250.5	.24	198	115.9	212.34	98.9	.51

Table 6-4 Association between vitamin level of participants with anxiety symptoms at week 8-12 and week 24-28 of pregnancy:

			at week 8-12 of pregnancy					at week 24-28 of pregnancy				
Variables			**Non-anxiety** (EPDS-3A ≤ 5) (n=227) (75.2%)		**Anxiety** (EPDS-3A ≥ 6) (n=75) (24.8%)			**Non-anxiety** (EPDS-3A ≤ 5) n=201(76.6%)		**Anxiety** (EPDS-3A ≥ 6) n=71 (23.5%)		
	Median	***Interquartile range***	***Median***	***Interquartile range***	***Median***	***Interquartile range***	***p***	***Median***	***Interquartile range***	***Median***	***Interquartile range***	***p***
Vitamin D	68.9	52.9-68.9	69.24	53-82.7	68.63	52.6-84.5	.64	69.44	27.79	68.67	33.89	.52
Folate	44.4	35.9-44.4	43.7	35.9-45.4	45.4	37.9-45.4	.36	44.44	10	44.06	11	.29
Vitamin B12	203.8	153.1-264.1	208	155.6-265.6	188	137.7-258.3	.16	202	111.95	209	104.1	.18

Table 6-5 Logistic regression analysis of risk factors for depression symptoms and vitamins levels among pregnant Omani women in the study Odds Ratio (95% CI):

	at weeks 8-12 of pregnancy:				at weeks 24-28 of pregnancy:			
Vitamin levels	**Unadjusted Model**		**Adjusted Model ***		**Unadjusted Model**		**Adjusted Model ***	
	OR (95% C.I.)	***p***	**OR (95% C.I.)**	***p***	**OR (95% C.I.)**	***p***	**OR (95% C.I.)**	***P***
Vitamin D	1.001(0.99-1.013)	0.793	0.995(0.98-1.011)	0.529	1.001(.988-1.015)	.866	.996(.98-1.013)	0.666
Folate	0.995(0.96-1.031)	0.794	0.995(0.943-1.051)	0.866	1.003(.962-1.047)	.875	1 (.948-1.057)	0.97
Vitamin B12	0.99(0.996-1.001)	0.277	0.99(0.995-1.003)	0.588	.998(.995-1.002)	.347	.99(.995-1.003)	.069
* a Variable(s) entered on step 1: Fish portion intake in one week by Category, Total DHA (mg) intake, Total EPA (mg) intake, Age Categories, Educational Level, Occupational Status, Annual Household Income, Housing tenure, Duration of Marriage, Whether the pregnancy was planned? History of Depression, Family History of Depression, Physical Activity, Parity, Obstetric Complication Previous Pregnancy, Obstetric Complication Current Pregnancy. BMI								

Table 6-6 Logistic regression analysis of risk factors for Anxiety symptoms and vitamins levels among pregnant Omani women in the study Odds Ratio (95% CI):

	at weeks 8-12 of pregnancy:				at weeks 24-28 of pregnancy:			
Vitamin levels	**Unadjusted Model**		**Adjusted Model ***		**Unadjusted Model**		**Adjusted Model***	
	OR (95% C.I.)	**p**	**OR (95% C.I.)**	**p**	**OR (95% C.I.)**	**p**	**OR (95% C.I.)**	**P**
Vitamin D	1.05 (0.993-1.016)	0.435	1.004(0.99-1.017)	0.614	1.004(0.992-1.016)	0.513	0.99(0.98-1.014)	0.756
Folate	1.01(0.971-1.05)	0.612	1.024(0.974-1.07)	0.355	0.999(0.962-1.037)	0.953	1(0.947-1.058)	0.974
Vitamin B12	0.99(0.995-1.001)	0.115	0.99(0.994-1)	0.088	0.999(0.996-1.002)	0.428	1(0.996-1.004)	0.974
* a Variable(s) entered on step 1: Fish portion intake in one week by Category, Total DHA (mg), Total EPA (mg), Age Categories, Educational Level, Occupational Status, Annual Household Income, Housing tenure, Duration of Marriage, Whether the pregnancy was planned? History of Depression, Family History of Depression, Physical Activity, Parity, Obstetric Complication Previous Pregnancy, Obstetric Complication Current Pregnancy. BMI								

6.4.3 Vitamin D, vitamin B12 and folate levels with pregnancy outcomes

Table 6-7 shows the relationship between median vitamin levels and pregnancy and neonatal outcomes among the women in the study. The median vitamin level was not associated with pregnancy and neonatal outcome variables, including GDM, PE and spontaneous preterm birth. However, as shown in

Table 6-7, there was a significant link between vitamin D levels and PE ($p = 0.04$) after we controlled for the vitamin supplement factors, and re-assessed the association between levels of vitamin D in women who were not taking supplements.

Table 6-8 summarises the relationship between median vitamin levels and neonatal birth outcomes (gestational duration (days) and customised birthweight centile in linear regression analyses). The linear regression analyses indicate no significant association between gestational period or customised birthweight centile with median vitamin levels.

Table 6-7 Association between vitamins levels and pregnancy and neonatal outcomes of the Omani pregnant women in the study who is not tacking vitamins supplements:

	Folate		**Vitamin B12**		**Vitamin D**	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Spontaneous preterm						
Healthy control group	-		-		-	
Preterm	1.01 (0.92-1.10)	0.90	1.00 (0.99-1.00)	0.45	0.99 (0.96-1.02)	0.64
GDM						
Healthy control group	-		-		-	
Women with GDM	1.04 (0.98-1.10)	0.16	1.00 (1.00-1.01)	0.62	0.99 (0.97-1.01)	0.18
Preeclampsia PE						
Healthy control group	-		-		-	
Women with PE	1.05 (0.90-1.22)	0.55	1.00 (0.99-1.01)	0.99	0.95 (0.90-1.00)	**0.04**

Table 6-8 Liner regression investigated the association between maternal fatty acids intakes with Gestational duration (days) and customised birthweight centile:

Gestational Duration (days)				**Customised birthweight centile**			
	β	95%CI	p		β	95%CI	p
Vitamin D	-0.514	-5.809 to 4.781	0.849	Vitamin D	-0.09	-0.29 to 0.049	0.163
Folate	0.032	-.285 to 0.348	0.843	Folate	-0.068	-0.59 to 0.454	0.798
Vitamin B12	-0.022	-.047 to .002	0.07	Vitamin B12	-0.036	-0.077 to 0.006	0.093

6.5 Discussion

This is the first study to examine the relationships between maternal concentrations of serum vitamin D, vitamin B12 and folate levels, depressive and anxiety symptoms, and maternal and neonatal outcomes among sample of pregnant Omani women.

6.5.1 Vitamin D, vitamin B12 and folate levels

According to the study, approximately 19.9% of the women participants had vitamin D deficiency, while 17% had vitamin B12 deficiency. Over the past twenty years, research has revealed significant maternal vitamin D deficiency in Oman. The micronutrient status survey conducted in 2004 shows that 21.4% of the women of childbearing age included in the study were deficient in vitamin D (<27.0 nmol/L) (525). A study including 103 pregnant Omani women, showed that 33% of the women had a vitamin D deficiency (526). Furthermore, in a study involving 41 pregnant Omani women, reported that all the women had a vitamin D deficiency (527). In 2017, the last National Nutrition Survey in Oman showed that 43% of women of childbirth age had vitamin D deficiency, while deficiencies in vitamin B12 and folate were reported at 11.6% and 8.9%, respectively (524).

6.5.2 Vitamin D, vitamin B12 and folate levels with depression and anxiety symptoms

This cohort study of sample of pregnant Omani women shows no association between vitamin D, vitamin B12, and folate levels with anxiety and depressive symptoms during pregnancy. This finding supports those of previous studies, which found no correlation between vitamin D levels and maternal depression. A survey of 498 US women found no association between maternal anxiety and depression and levels of vitamin D (399). In Denmark, Nielsen et al. discovered no significant association linking maternal

vitamin D levels and postnatal depression (408). However, this study collected data on depression from women who used anti-depressants within a year of giving birth. Furthermore, a study in Australia indicated no link between vitamin D levels in cord blood at childbirth and postnatal depression (409). A cross-sectional study of 130 women in Taiwan showed no significant links between vitamin D levels and postpartum depression (796). In contrast to our findings, numerous studies have shown an association between antenatal anxiety and depression and maternal levels of vitamin D (260,400,403,417). Furthermore, this study indicates no relationship between vitamin B12 and folate levels with depressive or anxiety symptoms during pregnancy among the study sample. This finding is inconsistent with other studies that show the same result with regard to prenatal depression (478,481,496,498). In contrast, other findings report a relationship between vitamin B12 and folate levels and anxiety and prenatal depression (247,496,497).

There could be various reasons why our findings differ from those of previous studies, such as varying strengths of association. The mixed results from studies examining the link between vitamin D, vitamin B12, and folate levels and maternal depression could be attributed to differences in outcome measurement methods, including differences in blood sample analysis, vitamin D sufficiency cut-off points, timing and assessment of anxiety depression symptoms, study structure, and control of potential confounders.

A range of different assessment tools were used to determine symptoms of depression and anxiety in previous studies. It could be the case that these instruments may measure other constructs. Confounding factors could explain the absence of such a relationship in this study. Nonetheless, we made a conscious effort to account for

numerous confounders based on previous research in the field. Thus, it is challenging to pinpoint an unmeasured variable that may account for our findings. Furthermore, variations in the data of the study sample could explain the variations in the relationships observed. For instance, Cassidy et al, (400), in their study of African American women, showed a significant prevalence (82.6%) of vitamin D deficiency. The Omani women in our study had a notably lower rate of vitamin D deficiency, at 19.9%. Vitamin D's correlation with mood/anxiety disorders may vary based on study population characteristics, leading to differing results between studies.

6.5.3 Vitamin D, vitamin B12 and folate levels with pregnancy outcomes

In this cohort study of sample of pregnant Omani women, we found no association between vitamin levels measured at early pregnancy (at weeks 8 – 12 weeks) with pregnancy and neonatal outcomes, including GDM, PE, spontaneous preterm birth, gestational age and customised birthweight centile. However, after re-analysis of the relationship linking vitamin D levels in women who were not taking vitamin D supplements, we found a significant association linking levels of vitamin D and PE. This conclusion may be because a large percentage of the women participating in the study (42%) informed that they were taking multivitamin supplements during pregnancy.

In 2008, Oman's Ministry of Health implemented a new standard for antenatal care, which requires a minimum of six visits for low-risk pregnancies. Each pregnant woman was encouraged to take a multivitamin dietary supplement (514). According to the 2017 National Nutrition Survey in Oman, 41% of pregnant women incorporated vitamin supplements into their diet, while 46% used B12 and folate supplements (524). Furthermore, a recent study in Oman indicates that roughly 34% of women consume

multivitamin supplements in their childbearing years (797). According to the 2017 National Nutrition Survey in Oman, a significant percentage of pregnant women (41%) incorporated vitamin supplements into their diet, while 46% used B12 and folate supplements (524). Furthermore, a recent study in Oman indicates that roughly 34% of women in their childbearing years consume multivitamin supplements (797).

Several RCTs have investigated vitamin D supplementation in reducing the incidence of PE. While a limited number of clinical trials have shown promising results, more research is needed to support these conclusions (361,425,464), while other attempts to decrease the occurrence of PE have not been successful in finding a positive solution (421,430,446,465–467). It is possible that the intervention studies yielded negative results because the intervention occurred too late in the pregnancy. This is because the protective effects of vitamin D are believed to start in early pregnancy (430).

PE is a complex condition with various pathophysiologies, one of which includes an intense inflammatory response (450). The correlation between vitamin D deficiency and PE needs to be better explained. Various studies suggest that vitamin D regulates a pregnant woman's immune response to the fetal-placental unit (451). Research suggests that vitamin D regulates cardiovascular reactions and immunomodulatory pathways. As a result, a deficiency in vitamin D might be linked with PE (452,453). Several observational analyses (454–457) and meta-analyses (458,459) have suggested that expectant mothers with insufficient levels of vitamin D could be at a higher risk of developing pre-eclampsia.

6.6 Conclusion

This is the first study to examine the relationships between serum vitamin D, vitamin B12 and folate concentrations and levels and depressive and anxiety symptoms and maternal and neonatal outcomes among sample of pregnant Omani women. This cohort study of a sample of pregnant Omani women shows no association linking vitamin D, vitamin B12, and folate levels with anxiety and depressive symptoms during pregnancy. There is no association between the vitamin levels measured and pregnancy and neonatal outcomes, including GDM, PE, spontaneous preterm birth, gestational age, and customised birthweight centile.

The current study has revealed a notable correlation between inadequate vitamin D levels and PE in women who do not consume vitamin D supplements. More comprehensive studies are needed to determine the connection between pregnant Omani women's vitamin levels and their depressive and anxious symptoms, as well as maternal and newborn outcomes.

Chapter 7: Final Summary and Discussion

7.1 Principal findings

Prenatal depression, also called antenatal depression, is a clinical type of depression that pregnant people experience during pregnancy. This condition is a type of mood disorder that can have an adverse impact on a woman's emotional and physical health, as well as the health of her unborn child (798,799). During pregnancy, a person may experience symptoms of depression that are similar to those of major depressive disorder. These symptoms may include ongoing sadness, hopelessness, emptiness, and a lack of interest or enjoyment in previously pleasurable activities. Some additional indications of this condition may involve alterations in eating habits or weight, disruptions in sleep patterns like insomnia or excessive sleepiness, tiredness or lack of energy, sentiments of unworthiness or excessive remorse, challenges with focusing or making decisions, and thoughts of self-harm or suicide in severe cases (800).

Emerging epidemiological surveys have suggested that prenatal depression is a worldwide public health problem due to its recurrence, its effects on pregnancy and non-mental outcomes, and its significant cost to the community (29,618). It negatively affects the health status of the mother and child development (619). It is one of the most common problems that affects pregnant women across the globe (19). Although the incidence of antenatal depression differs between countries, it is usually more prevalent than postpartum depression (48,620). Maternal depression is understood to be multifactorial rather than the result of a particular causal factor (31). It is identified as a complex psychiatric disorder with a multidimensional phenotype and includes social/psychological factors and biological aspects of pregnant women (32,33).

Psychiatric medications can be useful in managing mental health conditions in pregnant individuals. However, its use carries potential risks (799). Studies have indicated that pregnant women should be cautious when taking these medications (534). Certain psychotropic medications have been associated with a higher risk of congenital disabilities, LBW, and other harmful outcomes for the developing baby (62). Due to the fact that pregnant women are not recommended to be treated with psychiatric medications, there is often limited data on the safety of psychiatric medications during pregnancy, as pregnant individuals are often excluded from clinical trials. Furthermore, some medicines can cause withdrawal symptoms in newborns if used during pregnancy (62). Therefore, an alternative approach is needed to address psychiatric disorders during pregnancy (3).

During pregnancy, the dietary requirements for fetal development and to meet the metabolic and physiological needs of the mother are extensive (247). A balanced diet during fetal development is crucial. The recommended daily intake is approximately 71 g/day of protein and 175 g/day of carbohydrates, which provide between 45% and 65% of the total energy and 20-35% of total daily calories from healthy fats (801). Pregnancy is associated with a higher demand for omega-3 FAs (710), particularly during the third trimester, to meet biological needs and ensure fetal development (711). In the late stage of pregnancy, the fetal requirement for DHA is estimated to be approximately 50-70 mg/day to ensure the growth of the fetal brain (248). This is important because supersaturated FAs are regulated and selectively shifted to the fetus through the placenta (110,249). The development of the fetus requires DHA and arachidonic acid (AA) of omega-6 FAs; These FAs are critical structural building blocks of the fetal brain (121). Consequently, some pregnant women will be at significant risk of omega-3 deficiency and will have little or no DHA left over to meet their biological

needs (249). Consistent with this, maternal plasma DHA levels gradually decline, even with a balanced diet (253). Depletion of maternal omega-3 FAs could increase the incidence of antenatal depression (255–257).

Numerous studies have investigated the potential benefits of omega-3 FAs in treating mental disorders such as depression (534). Based on the findings of previous research (277,699), using omega-3 FAs as a form of treatment for psychiatric disorders has positive results. This provides hope for a more logical and effective approach to treatment.

Recent evidence has shown a relationship between omega-3 FAs bioavailability in neurological disorders, including depression (237). The lack of omega-3 FAs is associated with various pathological conditions, including antioxidant system deficiency (203). Low omega-3 FAs were also reported in erythrocyte membranes in depressed patients (700,701) and in women with a high risk of maternal depression (254,259). Therefore, adequate DHA intake during pregnancy is crucial for the fetus and healthy neural growth of the newborn (117). This has been hypothesised due to inadequate omega-3 FAs or a yet-to-be-charted genetic abnormality (567). In fact, several researchers have indicated that pregnant women with low omega-3 FAs are at significant risk of prenatal and postnatal depression and have recommended higher intakes (26,117,704). Furthermore, research on the link between omega-3 FAs intake (261,265,712,713) and erythrocyte levels (256,263,274,712,714) with prenatal depression and anxiety have produced inconsistent results. Current observational studies and RCTs have evaluated the potential effects and benefits of omega-3 FAs consumption in maternal depression (260). However, the exact association has not yet been determined (117).

Despite significant evidence that omega-3 FAs supplementation during pregnancy can reduce the incidence of maternal depression and improve pregnancy outcomes, many gaps in the evidence persist. This thesis serves to establish information on the incidence of anxiety and depression symptoms and pregnancy outcomes among pregnant Arabic-speaking women and to evaluate the potentially valuable role of omega-3 FAs from seafood intake and FAs levels in prenatal depressive and anxiety symptoms and pregnancy outcomes. This study aims to address most of the weaknesses of previous studies and fill a gap in our knowledge of maternal anxiety and depression and pregnancy and newborn outcomes with nutrition research in the case of a sample of pregnant Arabic women.

7.1.1 Chapter 3 - Depressive and anxiety symptoms and pregnancy outcomes among Omani pregnant women.

This is the first study to attempt to estimate the incidence and predictors associated with depression and anxiety symptoms among sample of pregnant Omani women. The study results showed that the incidence of prenatal depression was 29.8% at 8-12 weeks and 30.5% at 24 – 28 weeks, and the proportion of women who showed anxiety symptoms was 24.8% between 8 and 12 weeks of pregnancy and 26.1% between 24 and 28 weeks. The findings indicated that maternal depression and anxiety symptoms were higher than other estimates carried out in Oman. The level of education, history of depression, type of accommodation, and unplanned pregnancy were significantly associated with antenatal depression and physical activity, a predictor of high anxiety and depression symptoms among the study sample. The study results also showed that antenatal depression at 24-28 weeks of gestation is significantly related to GDM. Women with depression symptoms at 24-28 weeks of pregnancy had a 2.15-fold higher chance of developing GDM (GDM) during pregnancy

(95% CI: 1.02 – 4.52, p = 0.04) compared to women without depression symptoms during pregnancy. The incidence of maternal anxiety and depression was associated with a reduction in gestational age by one week at the time of delivery. The highest probability of an earlier delivery was observed in women with anxiety symptoms at 24-28 weeks of pregnancy, almost double compared to women with lower anxiety symptoms. There was no association between PE or spontaneous preterm and maternal depression and anxiety symptoms.

7.1.2 Chapter 4 - Intake and levels of maternal FAs and the presence of depressive and anxiety symptoms

This is the first study to examine associations between maternal FAs intake, maternal erythrocyte FAs profile, and anxiety and depressive symptoms in pregnant women in Oman. The study shows a low intake of fish in sample of pregnant Omani women. Around 20% of the study sample reported not eating fish, and about 39% reported limiting their fish consumption to less than two portions per week. The results of this prospective cohort study show a significant relationship between fish and omega-3 FAs intake and depressive and anxiety symptoms at weeks 8-12 and 24-28 of pregnancy. Significant associations remained even after controlling for potential confounders. These findings support previous work suggesting that fish and omega-3 FAs intake can offer protection against antenatal depression, specifically DHA, which is associated with reduced depressive and anxiety symptoms in pregnant women (260–262). In the present study, women with antenatal depression or anxiety symptoms during pregnancy had a lower erythrocyte concentration of arachidonic acid (20:4 n-6), total omega-6 FAs, docosahexaenoic acid (22:6 n-3), docosapentaenoic acid (22:5 n-3), eicosapentaenoic acid (20: 5 n-3), total omega-3, omega-3 index, and (AA+DHA)/MUFAs, but a higher omega-6/omega-3 ratio compared to healthy women.

Other FAs can be associated with depression, but few studies have explored this association. Interestingly, palmitic acid (16:00), lignoceric acid (24:00), and total SFAs were elevated in women with more symptoms of depression and anxiety. These findings did not change after adjusting for potential confounders (annual income, educational level, and history of depression factors). Furthermore, adrenic acid (22:4 n-6) and eicosatrienoic acid (20:3 n-3) were significantly associated after adjustment for potential confounders. Studies on the relationship between FAs levels and depression have focused on omega-3 FAs, specifically DHA and EPA. This study highlights the potential benefit of omega-3 FAs supplementation for both mothers and children and provides evidence to support a significant public health initiative.

7.1.3 Chapter 5 - Fish and Omega-intake and FAs levels and pregnancy outcome

This study investigates the transfer of FAs from the maternal circulation to the fetal circulation across the human placenta and supports the existence of a 'biomagnification' process of FA transfer. This study also examined the relationship between maternal nutrition, intake of FA and the profile of maternal FAs of erythrocytes and maternal and neonatal outcomes among a sample of pregnant Omani women. The study showed a significant association between fish and omega-3 FAs intake and GDM, and higher DHA intake is significantly associated with longer gestational age. Furthermore, EPA and DHA intake were positively associated with the customised birthweight centile.

This study has revealed the intricate mechanism of the placenta's active selection of specific FAs through biomagnification while rejecting other FAs through bioreduction. Biomagnification through the placenta is primarily regulated by AA and its allies, DGLA, ADA, and omega-6 DPA. Stearic acid is also biomagnified, probably to offer

the sn-1 position in membrane production (770). On the contrary, there is a bioreduction of MUFAs, specifically oleic acid and linoleic acid, to omega-6 and all omega-3 precursors DHA and EPA. Although DHA is biomagnified, the amount transferred from the mother to the fetus is relatively low compared to AA. Furthermore, the AA precursor, linoleic acid, is processed reversely, with its erythrocyte level in the fetus being half that observed in maternal RBCs. The fetus rejects it by bioreduction and then sends it to the mother's circulation through the umbilical arterial cord (771). This applies to all precursors of DHA, including α-linolenic acid and EPA, which are refused by the fetus, as previously found (110). Furthermore, this study shows that the placenta biomagnifies saturated FAs, and that MUFAs, including oleic acid, are bioreduced. Prenatally, saturated FAs are exchanged for MUFAs. The most probable explanation for these results is that the saturated FAs are shifted to be esterified beside the LCUFAs to cell membrane phosphoglycerides (121).

This cohort study showed that women with GDM had an elevated proportion of saturated FAs, omega-6 FAs, and omega-6/omega-3 FAs ratio and lower levels of omega-3 FAs, omega-3 index, and AA compared to the control group. The current study showed that women with PE had a lower level of DHA, total omega-3, and omega-3 index but a higher omega-6/omega-3 ratio than healthy women in the control group.

In this study, women in the spontaneous preterm birth group had significantly higher levels of the omega-6/omega-3 ratio, and the level of DHA, EPA, total omega-3 FAs and omega-3 index was lower among spontaneous preterm women than among healthy women in the control group. Women with spontaneous preterm labour had markedly elevated levels of MUFAs, particularly oleic acid. The findings Ogundipe et al, indicated a correlation between higher levels of oleic acid or MUFAs in red blood

cells from mothers at 12 weeks of pregnancy and a higher risk of premature birth and smaller size. his outcome could indicate the importance of the correct FA profile for the optimal outcome of pregnancy. The relationship between oleic acid and MUFAs can significantly impact metabolism, indicating a potential lack of essential FAs in the diet (121). In terms of essential FAs deficiency, the body attempts to maintain unsaturation in membrane lipids by substituting them with oleic acid and its long-chain unsaturated derivatives.

7.1.4 Chapter 6 - Vitamin D, Vitamin B12, and Folate and prenatal depression and anxiety symptoms

This is the first study to examine the relationships between maternal concentrations of serum vitamin D, vitamin B12, and folate levels, depressive and anxiety symptoms, and maternal and neonatal outcomes among sample of pregnant Omani women. The study results show that 19.9% of pregnant women had vitamin D deficiency, while 17% had vitamin B12 deficiency. This is lower than the previous estimate in Oman (524,527). This conclusion may be due to the fact that a large percentage of women who participated in the study (42%) reported taking multivitamin supplements during pregnancy. This cohort study of sample of pregnant Omani women showed no association between vitamin D, B12, and folate levels and anxiety and depressive symptoms during pregnancy. There is no association between measured vitamin levels and pregnancy and neonatal outcomes, including GDM, PE, spontaneous preterm birth, gestational age, and customised birth weight centiles. However, we found a significant relationship between low vitamin D levels and PE in women who did not take vitamin D supplements.

The aforementioned discussion points out novel studies that shed light on the importance of maternal mental health, FAs intake, and vitamin levels during

pregnancy. The findings provide important information to healthcare professionals in Oman. They highlight the importance of promoting a healthy lifestyle, adequate nutrition, and mental well-being among expectant mothers for the sake of maternal and neonatal health.

7.2 Originality and Contributions to Scientific Knowledge:

The study assesses the relationship between antenatal depression and anxiety and maternal nutrition, specifically omega-3 FAs intake and levels, among Arabic-speaking women in Oman. The PhD thesis investigates the influence of this association in a cultural context where depression is often attributed to supernatural causes. The study proposes nutritional interventions as adjunct treatments for mental health disorders, challenging conventional pharmacological approaches.

The research integrates longitudinal cohort analysis with dietary assessments and biochemical measurements, contributing to scientific knowledge. Using validated tools to estimate FAs intake and prenatal depression and anxiety symptoms, the study provides compelling evidence linking maternal nutrition to pregnancy outcomes and mental health. Examining erythrocyte FAs further enhances understanding of the physiological mechanisms underlying these associations.

In conclusion, the thesis represents a significant advancement in maternal and child health research, offering valuable insights to enhance perinatal care and address cultural determinants of maternal well-being.

7.3 Recommendation

Given the relatively high prevalence of depression and anxiety symptoms among pregnant Omanis in the present study, the Oman Ministry of Health should plan to apply routine screening for the presence of prenatal depression and anxiety symptoms

as a part of routine maternal care services to meet this clear health need and improve pregnancy outcomes. The early diagnosis of women with prenatal depression would allow healthcare professionals to offer mental support to these women and theoretically decrease the rate of mental health disorders and their related complications in Oman. In addition, women with antenatal depression can receive treatment, such as referrals to outpatient psychiatry services, depending on the severity of their condition. This can help mitigate the adverse effects of depression through early identification and management. Women who experienced poor pregnancy outcomes may also have suffered from antenatal depression. Therefore, screening pregnant women for depression and providing treatment and clearer referral systems could improve pregnancy and neonatal outcomes.

In the case of Oman, a country undergoing rapid change and development, raising awareness of healthy dietary patterns and lifestyles for pregnant women in Oman is essential. Confirmation of the results of the study could help healthcare policy makers in Oman establish guidelines and recommendations for an increased intake of omega-3 derived from oily fish in the case of pregnant women and fortification of food with omega-3 in different products, targeting other age groups. In Oman, no recommendations have been made regarding fish consumption during pregnancy; but in other countries increased fish and seafood intake or omega-3 FAs supplementation are highly recommended for women during pregnancy to ensure the well-being of the mother and fetus.

7.4 Strengths and limitations of the study

One significant advantage of this study is that it is the first to include a substantial group of participants (302 pregnant women) in whom maternal and cord erythrocyte

FA levels were measured and related to pregnancy outcomes. Furthermore, relevant descriptive factors were included in the study, allowing correction for confounding variables. The study also measured RBC FA levels in the early stages of pregnancy, providing valuable information. In addition, maternal FA intake was evaluated using a validated FFQ at 8-12 weeks of pregnancy. Erythrocytes are suggested to be a more consistent measure of long-term nutritional intake than plasma, which may reflect recent dietary intake (742). Again, FAs levels were reported as the percentage of FA in total FAs and as absolute quantities. Using the relative or absolute levels did not affect the results. This study includes early pregnancy reports and a longitudinal assessment of antenatal depression and anxiety symptoms at two evaluation points during pregnancy, ensuring that pregnancy outcomes did not influence the report. Studies on antenatal depression and anxiety symptoms that involve repetitive assessment and suitable statistical analyses are scarce. The entry and removal methods for the logistic regression model used in this study are effective as they involve repeated observations and monitor variations in FAs levels and depressive symptoms throughout pregnancy.

There are a few possible limitations to this research study. The study relied on self-reported questionnaires for data. This could have resulted in recall bias and unreliable estimates of the prevalence of antenatal depression and anxiety symptoms. The study lacked definitive diagnostic standards for antenatal depression. EPDS is primarily a screening tool and is not intended for diagnosis. All participants in the study will have a similar possibility of being misclassified regarding their status of results and exposure status. This could lead to bias with respect to the link between the outcome and exposure. Despite our efforts to account for potential confounder variables, there is still a possibility that unmeasured factors could have impacted our findings, leading

to residual confounders. The evaluation of omega-3 FAs intake is based on self-reported FFQ. As with other dietary assessment methods, these questionnaires allow for possible responder bias and inaccurate expectations about food portion sizes with respect to dietary intake. However, this was avoided in this study by using food model samples to estimate food portion sizes and validate omega-3 FAs FFQ with FAs levels in erythrocytes.

Furthermore, the methodology used to analyse FAs did not provide information on the specific type of FAs, such as whether it was a phospholipid, triglyceride, or cholesterol ester. However, the lipids observed were likely phospholipids because of the use of organic solvents to extract FAs from erythrocytes. Because of the large number of samples, it was not practical to use thin-layer chromatography. Although mass spectrometry would have been the optimal choice for separating different types of FAs, it is not currently available for use in our lab.

All participants in the study had a similar possibility of being misclassified with respect to their results and exposure status. This could lead to a bias in the power of the link between outcome and exposure. Due to its observational nature, unassessed outstanding factors can confound the work; for example, fish intake could function as a proxy for a healthy lifestyle. Therefore, potential confounders with seafood intake or depression were recorded and used as adjustment variables in the data analysis. Furthermore, antenatal depression is linked to various risk factors that fall under psychological, social, and biological categories (743,744), and distinguishing between these factors can be challenging. Certain aspects may have a more direct correlation with the condition, while others may act as causal factors. Adjusting for a non-relevant factor in a multifactorial disease could create new biases. We decided to use a convenient non-probability sampling procedure in a sample of pregnant women with

enough time and willingness to answer all interview questions. Non-randomised sampling restricts generalising about the population, as some of its features may not be well represented in the sample. Pregnant women who experience multiple responsibilities and face elevated stress levels may have hesitated to participate in the investigation.

7.5 Future work

This study has provided a valuable understanding of the relationship between maternal FAs intake and levels of depressive and anxiety symptoms and maternal and neonatal outcomes among sample of pregnant Omani women. However, due to the small sample size and several limitations mentioned in this chapter, these findings cannot be generalised to all pregnant Omani women.

In Oman and other Gulf countries with a similar language (Arabic) and culture, no available data or studies currently discuss the link between seafood consumption or omega-3 FAs intake and maternal depression and anxiety symptoms and pregnancy and neonatal outcomes. Therefore, more extensive studies are required to estimate the incidence of prenatal depressive and anxiety symptoms in Omani women and better assess the causality of the association. These future studies should use periodic diagnostic tests to accurately detect psychiatric disorders in expectant mothers to better confirm any potential causal link between depressive symptoms and other variables. Furthermore, we could not consider other variables or potential factors, such as genetic variations, which could have affected the erythrocyte profiles of FAs. More research is warranted to fully understand how omega-3 FAs affect other pregnancy outcomes including genetic factors and other potential mechanisms. Clinical trials on the impact of omega-3 FAs supplements during pregnancy, specifically on prenatal

and postnatal depression could be an essential step toward understanding the observed association. This study would clarify the potential benefit of omega-3 FAs supplementation for both mothers and children and provide the evidence necessary for a significant public health initiative.

Future research should also include a trial to correct FAs profiles prior to conception and throughout pregnancy. The trial will test omega-3 fish oil supplements, a mixture of trace elements and omega-3 FAs supplements, or nutritional interventions. This is important because the results of RCTs have been inconsistent possibly due to the populations studied, the supplements given and the timings of the studies, although recent clinical trials have shown a beneficial effect in reducing the preterm rate (308–311,802). The current study shows that FAs intake and levels among pregnant women are associated with depressive and anxiety symptoms and maternal and neonatal outcomes among sample of pregnant Omani women. Therefore, improving the status of FAs of women before conception could prevent adverse pregnancy outcomes. The potential impact of nutritional supplements or dietary interventions on pregnancy outcomes should be a top priority for pregnancy outcomes research.

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Appendix 1 Food-Frequency Questionnaire to Assess DHA and EPA intake

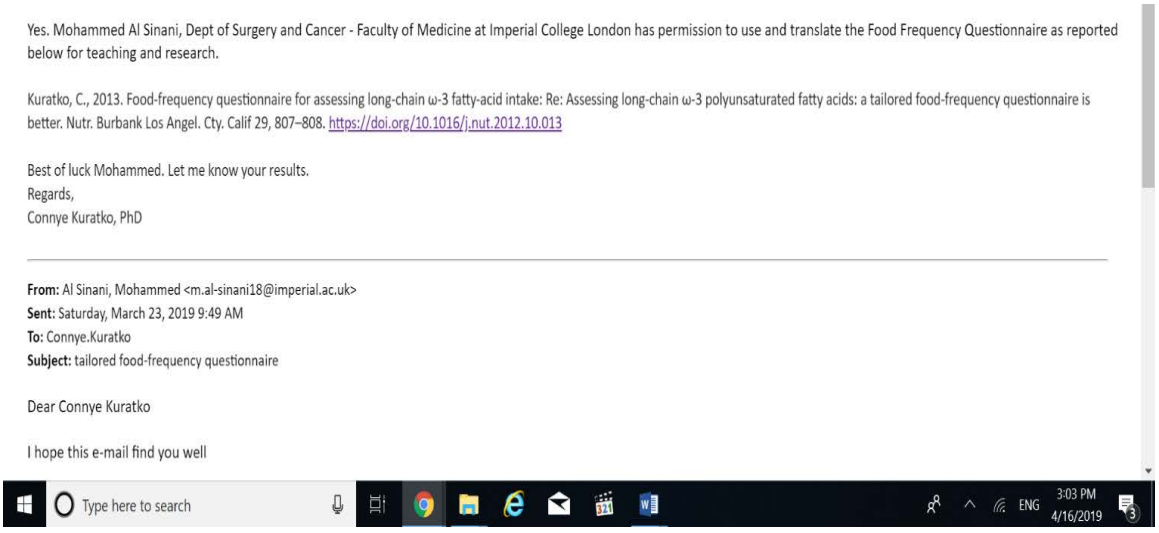
Depression and Anxiety in The Pregnant Omani Population in Relation to Their Fatty Acid Intake and Levels

Food-Frequency Questionnaire to Assess DHA and EPA Consumption

Question	Servings (no.)	DHA per serving (mg)	EPA per serving (mg)	Total DHA (mg)	Total EPA (mg)
1) How many 3-oz servings of the following fish do you eat monthly? ☐ Bluefish ☐ Sardines ☐ Salmon ☐ Pollock ☐ Herring ☐ Mackerel ☐ Whitefish ☐ Blue fin tuna ☐ Cisco, smoked		× 22	×14		
2) How many 3-oz servings of the following fish do you eat monthly? ☐ Bass ☐ Mussels ☐ Snapper ☐ Catfish ☐ Perch ☐ Sole ☐ Drumfish ☐ Redfish ☐ Trout ☐ Grouper ☐ Swordfish ☐ Flounder ☐ Shark ☐ Rockfish ☐ Tuna, canned (6-oz can)		×10	×5		
3) How many 3-oz servings of the following fish do you eat monthly? ☐ Carp ☐ Pike ☐ Clams ☐ Cod ☐ Haddock ☐ Pompano ☐ Fish sticks ☐ Lobster ☐ Crab ☐ Scallops ☐ Sturgeon ☐ Mullet ☐ Oysters ☐ Crayfish ☐ Fish patties/squares ☐ Shrimp (14) med		×5	×6		
4) How many 3-oz servings of liver (chicken, turkey or beef) do you eat monthly?		×7	×2		
5) How many egg yolks do you eat weekly (including egg yolks used in cooking)?		×3	× 0.25		
6) How many 3-oz servings of chicken, turkey, or other poultry (not including livers) do you eat weekly?		×5	×3		
7) How many 3-oz servings of red meat including beef and lamb do you eat weekly?		×10	×28		
8) How many 3-oz servings of fortified food with omega-3 do you eat weekly? Type……. Frequency……. d/wk/mo		mg/d	mg/d		
9) How many 3-oz servings of linoleic acid rich oils or fortified food with linoleic acid do you eat weekly?		mg/d	mg/d		

Type……. Frequency……. d/wk/mo					
10) Any u-3 dietary supplements (i.e., flax-seed oil, fish oil, neuromins)? Amount or strength……. Frequency…… d/wk/mo		mg/d	mg/d		
11) Any vitamin and mineral supplements (i.e selenium, magnesium, zinc, folic acid and vitamins B3, B6 and C)? Amount or strength……. Frequency…… d/wk/mo					
Totals					

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid
*All calculations are based on USDA Nutrient Database (USDA, Nutrient Database for Standard Reference release 14, 2002). y Instructions state: “Estimate your usual consumption of the following foods. Use the food model forms to help estimate portion sizes.” Questionnaire is administered by trained interviewer with the aid of food models.



Yes. Mohammed Al Sinani, Dept of Surgery and Cancer - Faculty of Medicine at Imperial College London has permission to use and translate the Food Frequency Questionnaire as reported below for teaching and research.

Kuratko, C., 2013. Food-frequency questionnaire for assessing long-chain ω-3 fatty-acid intake: Re: Assessing long-chain ω-3 polyunsaturated fatty acids: a tailored food-frequency questionnaire is better. Nutr. Burbank Los Angel. Cty. Calif 29, 807–808. https://doi.org/10.1016/j.nut.2012.10.013

Best of luck Mohammed. Let me know your results.
Regards,
Connye Kuratko, PhD

From: Al Sinani, Mohammed <m.al-sinani18@imperial.ac.uk>
Sent: Saturday, March 23, 2019 9:49 AM
To: Connye.Kuratko
Subject: tailored food-frequency questionnaire

Dear Connye Kuratko

I hope this e-mail find you well

Type here to search

ENG 3:03 PM 4/16/2019

Appendix 2 Edinburgh Postnatal Depression Scale (EPDS)

Depression and Anxiety in The Pregnant Omani Population in Relation to Their Fatty Acid Intake and Levels

EDINBURGH POSTNATAL DEPRESSION SCALE (EPDS)

We would like to know how you are feeling. Please UNDERLINE the answer which comes closest to how you have felt IN THE PAST 7 DAYS, not just how you feel today.

Here is an example, already completed.

I have felt happy:

Yes, all the time

Yes, most of the time

No, not very often

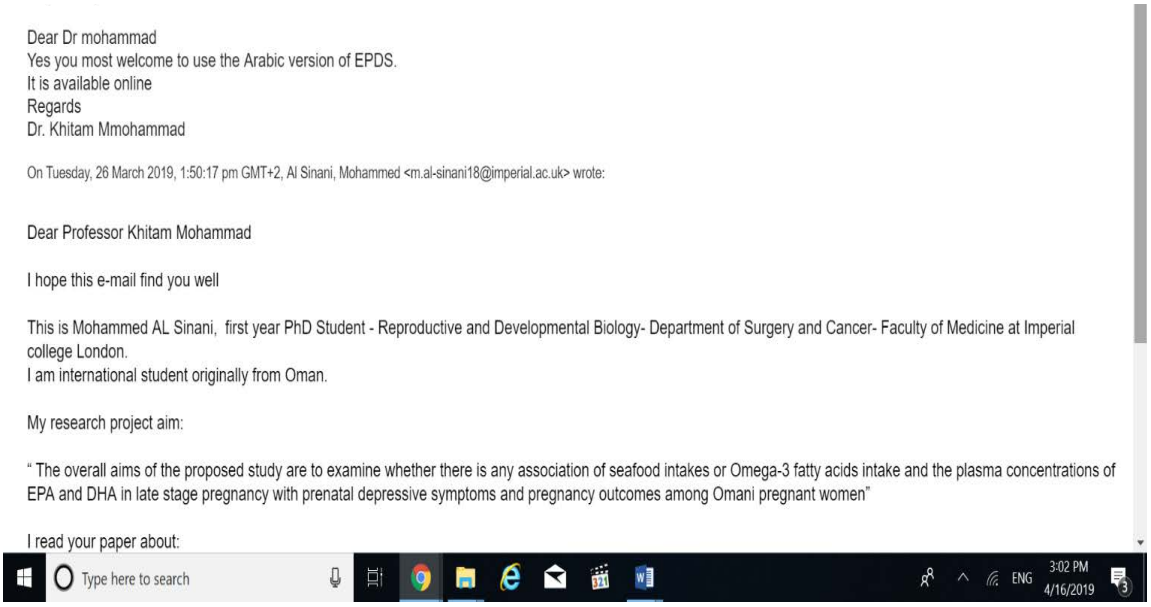
No, not at all

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past 7 days:

1. I have been able to laugh and see the funny side of things ➢ As much as I always could ➢ Not quite so much now ➢ Definitely not so much now ➢ Not at all	6. Things have been getting on top of me ➢ Yes, most of the time I haven't been able to cope at all ➢ Yes, sometimes I haven't been coping as well as usual ➢ No, most of the time I have coped quite well ➢ No, have been coping as well as ever
2.I have looked forward with enjoyment to things ➢ As much as I ever did ➢ Rather less than ➢ I used to Definitely less than I used to ➢ Hardly at all	7. I have been so unhappy that I have had difficulty sleeping ➢ Yes, most of the time ➢ Yes, sometimes ➢ Not very often ➢ No, not at all
3.I have looked forward with enjoyment to things ➢ As much as I ever did ➢ Rather less than I used to ➢ Definitely less than I used to ➢ Hardly at all	8. I have felt sad or miserable. ➢ Yes, most of the time ➢ Yes, quite often ➢ Not very often No, not at all
4.I have blamed myself unnecessarily when things went wrong ➢ Yes, most of the time ➢ Yes, some of the time ➢ Not very often ➢ No, never	9. I have been so unhappy that I have been crying ➢ Yes, most of the time ➢ Yes, quite often ➢ Only occasionally ➢ No, never
5. I have been anxious or worried for no good reason ➢ No, not at all ➢ Hardly ever	10 . The thought of harming myself has occurred to me ➢ Yes, quite often ➢ Sometimes

➢ Yes, sometimes ➢ Yes, very often	➢ Hardly ever ➢ Never



Dear Dr mohammad
Yes you most welcome to use the Arabic version of EPDS.
It is available online
Regards
Dr. Khitam Mmohammad

On Tuesday, 26 March 2019, 1:50:17 pm GMT+2, Al Sinani, Mohammed <m.al-sinani18@imperial.ac.uk> wrote:

Dear Professor Khitam Mohammad

I hope this e-mail find you well

This is Mohammed AL Sinani, first year PhD Student - Reproductive and Developmental Biology- Department of Surgery and Cancer- Faculty of Medicine at Imperial college London.
I am international student originally from Oman.

My research project aim:

" The overall aims of the proposed study are to examine whether there is any association of seafood intakes or Omega-3 fatty acids intake and the plasma concentrations of EPA and DHA in late stage pregnancy with prenatal depressive symptoms and pregnancy outcomes among Omani pregnant women"

I read your paper about:

Appendix 3 Wast management at CWH

Chelsea and Westminster Hospital NHS
NHS Foundation Trust

369 Fulham Road
London SW10 9NH

3rd September 2015

Our Ref: FOI 2015/342

Following your request for information under the Freedom of Information Act 2000 which we received on 20th July 2015, we are providing you information as held by Chelsea and Westminster Hospital NHS Foundation Trust.

In your query, you requested the following information with regards to our current recycling and waste support and maintenance contracts.

Examples of recycling contracts we could have:
- Green Waste Disposal
- Household Waste Recycling Centres
- Refuse Recycling Street Cleaning
- Recycling Collection Services

Examples of waste management contracts we could have:
- Waste Development Environmental Assessment
- Waste Transfer & MRF (Materials recovery facility)
- Waste Disposal Landfill
- Bulky Waste

For each of the types of contract above you requested:

1. Contract Type- From the examples given above please state what type of contract this is. Please state other and type of contract if the type of contract is not listed above. In some cases the organisation will have one or two big contracts that is covered in a managed contract please state in the contract description what services the contract provides as well.

   The only recycling the Trust currently do is Cardboard and Shredded Confidential paper. We have a contract through ISS Facility Services with Shred Station who come in once a week and collect the bails of cardboard and bails of Shredded Paper, at no charge to the Trust. We average 2.5 ton of cardboard a week and 1-2 ton of shredded paper. Shred Station return the bails back into the industry.

2. The supplier of the recycling or waste contract

   Our main waste contract for the trust is Tradebe who handle all our Clinical and Domestic Waste, the average monthly spend on waste through Tradebe is £31,000.00. Shred Station has a small contract to handle our Confidential Waste at one of our remote stations, with a monthly spend of £1200.00. All waste contracts are a sub-contract of the Soft FM provider ISS facility Services.

3. What is the annual average spends for each of the suppliers. For those organisations with new contracts can you please specify the estimated spend?

   For clinical and Domestic Waste through Tradebe our annual spend is c £370,000.00. For the small amount of Confidential Waste with Shred Station is c £12,000.00

Appendix 4 Ethical Approval

Sultanate of Oman
Ministry of Health
Directorate General of Planning and Studies

سلطنة عمان
وزارة الصحة
المديرية العامة للتخطيط والدراسات

Ref. : MoH/DGPS/CSR/PROPOSAL_ APPROVED/22/2019 الرقم :

Date 1.07.2019 التاريخ :

الموافق :

Mohammed Al-Sinani
Principal Investigator

Study Title : Depressive Symptoms in Pregnant Omani Women with Low Omega-3 Fatty Acid Intake

Proposal ID: MoH/CSR/19/9668

After compliments,

We are pleased to inform you that your research proposal **'Depressive Symptoms in Pregnant Omani Women with Low Omega-3 Fatty Acid Intake'** has been approved by the Research and Ethical Review & Approval Committee, Ministry of Health.

On completion of the study, you are required to provide a copy of the final report within 2 months to the Centre of Studies and Research.

Regards,

Dr. Ahmed Mohamed Al-Qasmi
Director General of Planning and Studies
Chairman, Research and Ethical Review & Approval Committee
Ministry of Health, Sultanate of Oman.

Cc
Day file

P.O. Box : 393, Postal Code: 100, Muscat
Tel.: 22357254, Fax : 22357260

E-mail: dg.plan16@gmail.com

ص.ب : ٣٩٣ ،الرمز البريدي : ١٠٠
مسقط ، هاتف: ٢٢٣٥٧٢٥٤، فاكس : ٢٢٣٥٧٢٦٠

Appendix 5 Sociodemographic and medical characteristics of the participants

Depression and Anxiety in The Pregnant Omani Population in Relation to Their Fatty Acid Intake and Levels

General health and lifestyle questioner

Sociodemographic and medical characteristics of the participants

1. Age:

2. Educational level

	None		Basic Education		Secondary Education		Degree or above

3. Occupational status

	Employed		Unemployed		Retired

4. Annual household income

	Less than 500 OR		500 OR – 1000 OR		1000 OR or above

5. Housing tenure

	Owner house		Rent		Other

6. Smoking

	Yes		No

7. Gestation (weeks)

8. Duration of Marriage

	Less than 5 years		Less than 10 years		More than or equal to 10 years

9. Gravidity

	Primigravida		Multigravida

10. Obstetric complication Current Pregnancy

	No		Yes, please specify …….…….

11. Obstetric complication Previous Pregnancy

	No		Yes, please specify …….

12. History of Miscarriage

	Yes		No

13. Mode of Delivery in previous pregnancies

	1st trimester (0-13 week)		2nd trimester (14-27 week)		3rd trimester (28-42 week)

14. Pre-pregnancy Body Mass Index (BMI) (kg/m2)

15. Current Body Mass Index (BMI) (kg/m2)

16. Gestational weight gain (GWG)

17. Physical activity

18. Anemia status (haemoglobin levels <11.0 gm/dL),

19. History of depression

20. Family history of depression,

21. whether the pregnancy was planned or spontaneous and marital conflict.

Pregnancy Outcome:

22. Gestation Age at delivery (weeks)

23. Normal or caesarean delivery

24. Preterm birth (preterm delivery)

25. Birth sex

26. Birth recumbent length

27. Birth weight kg

28. Head circumference

29. Apgar score

30. Fetal macrosomia

Appendix 6 Participant Information Sheet

Study Title: Depressive Symptoms in Pregnant Omani Women with Low Omega-3 Fatty Acid Intake

You are being invited to take part in a research study which is a part of a PhD project. Before you decide whether or not to take part, it is important that you understand what the research is for and what you will be asked to do. Please read the following information and do not hesitate to ask any questions about anything that might not be clear to you. Make sure that you are happy before you decide what to do. Thank you for taking the time to consider this invitation.

1. **Purpose of the study**

The study aims to evaluate whether low seafood intake and the Omega-3 fatty acids status in the late stage of pregnancy among Omani pregnant women is associated with increased prevalence of depressive symptoms and pregnancy outcomes. It is part of PhD research project being under taken by Mr Mohammed Al Sinani.

2. **Why have I been invited to take part?**

You have been chosen to participate in this study because you are able to speak and write in Arabic, you are an Omani pregnant woman in your third trimester of pregnancy, over 18 years of age, and have no known of psychiatric or neurological disorders that would interfere with given consent; expecting to continue prenatal health care in the Antenatal clinic at Al Buraimi Hospital.

3. **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep. You will also be asked to sign a consent form. If you choose to take part, you can change your mind at any time and withdraw from the study without giving a reason.

4. **What will happen to me if I take part?**

If you agree to take part in the study, you will be asked to give some information about yourself, e.g. with assistance of the researchers in the study you will need to complete two non-invasive questionnaires to assess your depressive symptoms during pregnancy by using the Edinburgh Postnatal Depression Scale (EPDS) and to assess your daily seafood and Omega-3 intake by using a semi-quantitative food-frequency questionnaire. You need to allow the investigators to take a blood sample (5ml) from you for the study purpose. The demographic data will be collected from participants or from the medical records as the researcher has access to the data.

5. **Risks and / or disadvantages?**

There are no risks in answering the questionnaire eliciting your emotional state or about your food intake or providing blood sample (5ml) for the study purpose However, it is possible that you may feel some degree of emotional discomfort when answering the

questions, a result of participating in this study, if this the case then you will be referred to the appropriate health care professional.

6. Are there any possible benefits in taking part?

There are no direct benefits to the you. However, by participating in this study, it will help us understand the association of seafood intakes or Omega-3 fatty acids intake and the red blood cell level of Omega-3 in late stage pregnancy with prenatal depressive symptoms and pregnancy outcomes among Omani pregnant women. This may help to develop an intervention to reduce antenatal depression among Omani patients and improve pregnancy outcomes.

7. What if something goes wrong?

If you have any complaints about the research you can contact the researcher who will try to help you to deal with your query. If you feel your query has not been handled to your satisfaction you can contact Professor Mark Johnson, Clinical Chair in Obstetrics, Faculty of Medicine, Department of Surgery & Cancer, Reproductive and Developmental Biology, Imperial College London, UK.

8. Will my taking part in this study be kept confidential?

All information you provide will be stored confidentially and will only be used for the research purposes of this study. All information collected will be given a number, so your information will be anonymously stored on a password-protected computer. Only the Principle Investigation (PhD student) and the research team will have access to the data. The final results of the research will be published, but all personal data will be completely removed prior to publication, as required under data legislation. However, if you withdraw from the study your data will be marked as 'missing data'.

9. What will happen to the results of the study?

The findings of this study will be written up as part of the researcher's PhD thesis. Additionally, findings may be published in healthcare journals and presented at national and international conferences, as well as being used for written publications in peer-reviewed journals. If you are interested in knowing the outcomes of this study, please contact the PhD student (please see below) who will provide a summary of the findings.

10. Who has reviewed this study?

This study has been reviewed and approved by the Professor Mark Johnson, Clinical Chair in Obstetrics, Faculty of Medicine, Department of Surgery & Cancer, Reproductive and Developmental Biology, Imperial College London, UK. In addition, the Ministry of Health in Oman has been reviewed and approved by the Research and Ethical Review and Approve Committee (RERAC(.

11. Contact details:

PhD Research Student (PI)	**(Co) Principle Investigator**	

Mr, Mohammed AL Sinani. PhD student Department of Surgery & Cancer, Reproductive and Developmental Biology, Imperial College London P.O. Box 8 P c: 512 Phone: + 968 25650855-258, Fax: + 968 25650767, Mobile: 968 95951666 E-mail: m.al-sinani18@imperial.ac.uk	Professor Mark Johnson, Clinical Chair in Obstetrics, Faculty of Medicine, Department of Surgery & Cancer, Reproductive and Developmental Biology, Imperial College London, UK +44 (0)20 8846 7887 mark.johnson@imperial.ac.uk	Professor Samir Al-Adawi, College of Medicine and Health Sciences, Department of Behavioral Medicine, Sultan Qaboos University, P.O. Box 35, Al-Khoudh 123, Muscat, Oman, Phone: + 968 2414 1139, Fax:+ 968 2441 5419, Mobile: 968 9938 0246 E-mail: adawi@squ.edu.om

Thank you for taking part in this research.

Appendix 7 Consent Form

Depression and Anxiety in The Pregnant Omani Population in Relation to Their Fatty Acid Intake and Levels

	Please Initial
• I confirm that I have been given and have read and understood the information sheet for the above study and have asked and received answers to any questions raised	[ ]
• I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my rights being affected in any way	[ ]
• I understand that the researchers will hold all information and data collected securely and in confidence and that all efforts will be made to ensure that I cannot be identified as a participant in the study (except as might be required by law) and I give permission for the researchers to hold relevant personal data	[ ]
• I understand that sections of any of my medical notes may be looked at by a (Mohammed Al Sinani) responsible person Individuals from Imperial College London or regulatory authorities (Ministry of Health in Oman) where It is relevant to my taking part in this research. I give permission for these individuals to Access my records that are relevant to this research.	[ ]
• I agree to take part in the above study	[ ]

Participant Name	**Signature**	**Date**

Researcher Name	**Signature**	**Date**
Mohammed AL Sinani		

Appendix 8 Material Transfer Agreement

MATERIAL TRANSFER AGREEMENT

(Imperial College London the receiver of the Material)

1. ALBuraimi Hospital, Ministry of Health in Oman
[ ] ("Supplier"), has collected and/or developed the materials known as

Insert description of materials: **Human Blood Sample for research purposes**
and includes any constructs, strains, progeny, derivatives, portions, improvements and components (as the case may be) obtained from or as a result of the use of the materials (the "Materials")

2. *Insert name and address of Scientist's Institution*: **Imperial College of Science, Technology and Medicine** incorporated by Royal Charter in the United Kingdom ("Imperial") the address of which is Exhibition Road, London, SW7 2AZ.

Insert name of Scientist: **Prof Mark Johnson**
(the "Recipient") who is an employee of Imperial

and wishes to acquire a sample of the Materials for academic research relating to:

Insert description of academic research for which Materials are to be used for: **Clinical Medicine Research (Metabolism, Digestion and Reproduction) (PhD) Mohammed AL Sinani, PhD Student – CID:01221969**
(the "Research Programme")

3. *Insert quantity of Materials to be supplied and period for which they are to be provided for*: The Supplier is willing to provide a sample of **Human Blood Sample** of the Materials for a period of **one** years (the "Term") on the Terms and Conditions shown overleaf, and the Recipient and the Institution agree to comply with those Terms and Conditions (the "Agreement")

AGREED by the parties through their authorised signatories:-

Appendix 9 Materials used in the methods:

1 _ Chemicals, reagents and solvents:

Reagents and solvents	Supplier
2,6-di-tert-butyl-p-cresol	Fisher Scientific
Acetyl chloride	ACROS Organics
Chloroform	Fisher Scientific
Methanol, HPLC Grade, ≥99.9%	Fisher Scientific
Methanol, anhydrous	Fisher Scientific
Ponceau-S solution	Fisher Scientific
Potassium hydrogen carbonate (KHCO3)	Fisher Scientific
Sodium chloride (NaCl)	Fisher Scientific
Sodium sulphate (Na2SO4), anhydrous	ACROS Organics
Heptane HPLC grade	Fisher Scientific
Petroleum Ether 60-80°C, Extra Pure, SLR,	Fisher Scientific
FAME standard supelco 37 mix	Merck

2_ Buffers and Solutions

5 % w/v NaCl (saline)	5 g NaCl per 100 ml dH2O
0.85 % w/v NaCl (saline)	0.85 g NaCl per 100 ml dH2O
2 % w/v potassium bicarbonate (KHCO3)	2 g KHCO3 per 100 ml dH2O
Chloroform + 0.01 % butylated hydroxyl toluene (BHT)	100 mg 2,6-di-tert-butyl-p-cresol per 1 L chloroform
Methanol + 0.01 % BHT	100 mg 2,6-di-tert-butyl-p-cresol per 1 L methanol
Chloroform/Methanol, 2:1 v/v, + 0.01 % BHT	500 mL chloroform + 0.01 % BHT with 250mL menthaol + 0.01 5 BHT
Petrol spirit + 0.01 % BHT	100 mg 2,6-di-tert-butyl-p-cresol per 1 L petroleum ether

Appendix 10 List of fatty acids and their respective common names

Fatty acids	Common Name
Saturated	
C16:0	Palmitic acid
C18:0	Stearic acid
C20:0	Arachidic acid
C22:0	Behenic acid
C24:0	Lignoceric acid
Mononunsaturated	
C16:1n7	Palmitoleic acid
C18:1n9	Oleic acid
C18:1n7	Vaccenic acid
C20:1n9	Eicosenoic acid
C22:1n9	Erucic acid
C24:1n9	Nervonic acid
Polyunsaturated	
C18:2n6	Linoleic acid
C20:2n6	Eicosadienoic acid
C20:3n6	Dihomo-y-linolenic acid
C20:4n6	Arachidonic acid
C22:2n6	Docosadienoic acid
C22:4n6	Adrenic acid
C22:5n6	Docosapentaenoic acid- 6
C18:3n3	Alpha-linolenic acid
C20:5n3	Eicosapentaenoic acid
C22:5n3	Docosapentaenoic acid- 3
C22:6n3	Docosahexaenoic acid

Appendix 11 Result Tables

Table:1 Linear regression analysis of the relation between maternal the FAs (absolute content) of maternal erythrocyte (mg/ml) and **customised birthweight centile** of the Omani pregnant women:

Maternal FA (mg/ml)				**Cord FA** (mg/ml)			
	β	**95%CI**	**p**		**β**	**95%CI**	**p**
∑SFA	-0.171	(-21.255-95.431)	0.014	18:3 ω3	0.172	(244.2-25.6)	0.02
18:1ω9	0.146	(-133.23--15.375)	0.035	AA/LA	0.179	(0.447-4.19)	0.015
18:2ω6	0.468	(11.988-335.594)	0.002				
∑ω3	-0.397	(145.165-646.995)	0.019				
AA/LA	0.491	(-534.551--49.261)	<.001				
ω6/ω3	-0.354	(9.423-33.168)	0.041				

Table 2: Linear regression analysis of the relation between maternal the FAs (absolute content) of maternal erythrocyte (mg/ml) and **gestational age (days)** of the Omani pregnant women:

Maternal FA (mg/ml)				**Cord FA** (mg/ml)			
	β	**95%CI**	**p**		**β**	**95%CI**	**p**
18:1ω7	-0.15	(-1117.93--76.527)	0.025	16:00	-0.86	(-380.994--15.573)	0.034
				18;00	-0.516	(-387.757--10.149)	0.039
				24;00	-0.315	(-655.704--1 2.907)	0.003
				∑SFA	0.939	(4.196-367.631)	0.045
				20:1ω9	-0.202	(-1106.691--181.392)	0.007
				AA/DHA	-0.521	(-5.549--1.996)	<.001
				ω6/ω3	0.451	(1.918-6.778)	<.001

Appendix 12 Copy Right

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Aug 10, 2023

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Order reference number	1
Portions	Figures. 1. Metabolism of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids in human brain.
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Appendix 13 Copy Right